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[Continued on next page]

(54) Title: GLUTX POLYPEPTIDE FAMILY AND NUCLEIC ACIDS ENCODING SAME

FIG. 1A	FIG. 1B
FIG. 1C	FIG. 1D
FIG. 1E	FIG. 1F
FIG. 1G	FIG. 1H

(57) Abstract: The present invention provides GLUTX1, GLUTX2 and GLUTX3, a family of novel isolated polypeptides, as well as a polynucleotides encoding GLUTX1, GLUTX2 and GLUTX3, and antibodies that immunospecifically bind to GLUTX1, GLUTX2 and GLUTX3, or any derivative, variant, mutant, or fragment of any one of the GLUTX family of polypeptides, polynucleotides or antibodies. The invention additionally provides methods in which the GLUTX family of polypeptides, polynucleotides and antibodies are used in detection and treatment of a broad range of pathological states, such as aberrant hexose transport, as well as to other uses.

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GLUTX POLYPEPTIDE FAMILY AND NUCLEIC ACIDS ENCODING SAME

FIELD OF THE INVENTION

The invention generally relates to novel nucleic acids and polypeptides and more particularly to novel nucleic acids encoding polypeptides related to glucose transporters.

BACKGROUND OF THE INVENTION

Glucose transport across biological membranes requires the presence of specific integral membrane proteins that, in mammals, fall into two classes. The first class includes the Na^+/glucose cotransporters, SGLT1 and SGLT2, which are present mainly in the apical membrane of epithelial cells from intestine and kidney. See, *e.g.*, Hediger and Rhoads 1994 Physiol. Rev. 74: 993-1026. The second class includes the facilitative glucose carriers GLUT1, GLUT2, GLUT3, GLUT4 and GLUT5 (referred to collectively herein as "GLUT1-5"), which are present in every tissue. See, *e.g.*, Mueckler 1994 Eur. J. Biochem. 219: 713-725; Gould and Holman 1993 Biochem. J. 295: 329-34. Much work has been devoted to the study of the GLUTs to determine their role in the control of glucose fluxes in different organs and their respective importance in the control of whole body glucose homeostasis. Key roles for these transporters involve glucose absorption into the body, glucose uptake by the brain, storage in liver, insulin-dependent uptake in muscles and adipocytes, and glucose sensing by pancreatic cells. See, *e.g.*, Thorens 1996 Am. J. Physiol. 270: G541-G553; Rea and James 1997 *Diabetes* 46: 1667-1677. Defects in any of these mechanisms may have profound pathophysiological consequences, in particular in the development of type 2 diabetes. See, *e.g.*, Kahn 1998 *Cell* 92: 593-596; Unger 1991 *Science* 251: 1200-1205.

Glucose (hexose) homeostasis requires the tight quantitative and temporal regulation of glucose flux into and out different organs. The brain constantly needs a fixed supply of glucose for its own energy production and this requires that circulating glucose never decrease below a minimal level of ~5mM. Glucose can enter the organism through the intestinal wall. It is also produced endogenously by the liver what glucose is stored as glycogen or produced by the gluconeogenic pathway. Absorption of glucose by muscle and adipose tissues in the postprandial state is a major component of glucose homeostasis and is required to prevent development of hyperglycemia.

These different glucose uptake and production mechanisms are under tight hormonal regulation. In particular glucagon and insulin, produced by the alpha and beta cells of the

Islets of Langerhans under hypoglycemic or hyperglycemic conditions, respectively, provide major control over these fluxes. It is however now established that other glucose sensor units present in different parts of the body participate in the fine control of glucose homeostasis. It is known for instance that the hypothalamus contains glucose-sensing or glucose responsive neurons, the electrical activity of which is under positive or negative control by hypo or hyperglycemia. These neurons participate in the control of food intake, activity of the autonomous nervous system and secretion of hormones from the endocrine pancreas or the adrenal gland. A need remains to identify other components of the glucose homeostasis pathways in order to better treat diseases and disorders related to glucose (hexose) control, such as, *e.g.*, hyperglycemia and hypoglycemia.

SUMMARY OF THE INVENTION

The invention is based in part on the discovery of a family of novel polypeptides, and nucleic acids encoding them, that are related to GLUT1-5 and GLUT8, and have hexose binding and or transport function. GLUTX1, GLUTX2 and GLUTX3 comprise a related family of GLUTX polypeptides, as shown in at least human, rat and mouse. Nucleic acid sequences and their encoded polypeptide sequences are provided for GLUTX1, GLUTX2 and GLUTX3. Consensus sequences for each GLUTX isoform, as well as the GLUTX generic family, are also provided in the invension. Conserved GLUTX polypeptide regions, and the nucleic acids encoding the same, are also provided. Consensus sequences provide the means to clone other members of the GLUTX family of proteins, in addition to the GLUTX sequences provided herein. Antibodies specific to GLUTX1, GLUTX2 and GLUTX3, and to a consensus GLUTX polypeptide, or fragments or derivatives thereof, are also described.

Structural domains of GLUTX include transmembrane domains 1-12 (TM1 to TM12), interdomain loop regions, an amino-terminal domain and a carboxy-terminal domain of the GLUTX polypeptides. A GLUTX amino-terminal domain and a carboxy-terminal domain are defined as the tail regions prior to the first TM domain and after the last TM domain, respectively. Regulatory sequences, including 5' UTR and 3' UTR, as well as methods of cloning promoter and enhancer regions of GLUTX genes, are described. A dileucine motif, present in GLUTX1, GLUTX2 and GLUTX3 polypeptides is required for regulating the localization of the expressed GLUTX protein. GLUTX polypeptides mutated to no longer contain the N-ter dileucine repeat are provided for strong expression of the respective GLUTX protein on the cell surface, rather than having expressed GLUTX polypeptides being sequestered intercellularly.

GLUTX polypeptides, nucleic acids and antibodies may be used to modify the regulation and expression of GLUTX polypeptides. Methods of treatment of disorders associated with aberrant hexose transport, including but not limited to ischemia, diabetes, hyperglycemia and hypoglycemia, are described using GLUTX polypeptides, nucleic acids and antibodies. Kits containing GLUTX polypeptides, nucleic acids and antibodies are also included. GLUTX polypeptides and nucleic acids may be isolated, contained in expression vectors, in host cells, or in transgenic animals.

GLUTX2 and GLUTX3 polypeptides are highly expressed in brain tissue, and are especially suited as targets in regulating ischemia and other hexose-related disorders in these tissues. GLUTX1 is highly expressed in testes, and is likewise a suitable candidate for treatments of hexose related disorders in these tissues.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 represents an alignment of GLUTX1 with *B. vulgaris* integral membrane protein (IMP) (Swiss-Prot accession number Q39416, GenBank accession number AAB53155), *E. coli* galactose proton symport (GALP) (Swiss-Prot accession number P37021), *L. brevis* xylose proton symport (XYLT) (Swiss-Prot accession number O52733), and rat GLUTs 1-5 (Swiss-Prot accession numbers P11166, P11168, P11169, P14672, and P22732, respectively).

FIG. 2 is a representation of the individual GLUTX proteins, wherein **Panel A** represents an alignment of GLUTX1 mouse, rat, and human amino acid sequences; and **Panel B** represents an alignment of GLUTX2 human and rat amino acid sequences.

FIG. 3 is a representation of the GLUTX family of polypeptides, wherein **Panel A** represents an alignment of human GLUTX1, GLUTX2 and GLUTX3 amino acid sequences;

and **Panel B** represents an alignment of rat GLUTX1, GLUTX2 and GLUTX3 amino acid sequences.

FIG. 4 is a graphic representation of the rat GLUTX1 sequence, putative domains, and the amino acid sequences used to construc fusion proteins with GST for antibody production.

FIG. 5 is a graphic representation of GLUTX functionality, wherein **Panel A** presents 2-DOG uptake in *Xenopus* oocytes injected with GLUTX1 wild-type (*WT*) RNA and GLUTX1(LL-AA) RNA compared with noninjected control oocytes; **Panel B** presents the effect of competitors and inhibitors on uptake of 2-DOG in GLUTX1(LL-AA)-injected oocytes; and **Panel C** presents a dose curve for uptake of 2-DOG in GLUTX1(LL-AA)-injected oocytes fitted using the Michaelis-Menten equation.

FIG. 6 is a graphic representation of the dose curve for uptake of myo-inositol in oocytes injected with 25 ng of GlutX2(RRR-AAA) RNA, fitted using the Michaelis-Menten equation.

DETAILED DESCRIPTION OF THE INVENTION

Glucose (hexose) homeostasis requires the tight quantitative and temporal regulation of glucose flux into and out different organs. Aberrant regulation of hexose transport in the body can lead to hyperglycemia, hypoglycemia, and the like. Other disease models, *e.g.*, diabetes and ischemia, also involve aberrant regulation of or responses to hexose levels in the blood and surrounding tissues. The brain constantly needs a fixed supply of glucose for its own energy production and this requires that circulating glucose never decrease below a minimal level of ~5mM. It has indeed been shown that stimulation of activity of different brain regions is associated by increased glucose metabolism, that ischemia leads to increase glucose utilization and that diseases of the central nervous system may be associated with impaired stimulation of glucose uptake by specific brain areas

Facilitative glucose transport, one of two mechanisms to regulate glucose in the body, is regulated by a family of proteins that include GLUT 1-5 and GLUT8. See, *e.g.*, Carayannopoulos *et al.*, 2000 PNAS 97: 7313-7318. Structurally, the GLUTs form a family of highly related hexose transport proteins that belongs to a larger sugar transport superfamily consisting of more than 133 members distributed in a wide variety of species. See, *e.g.*, Pao *et al.* 1998 *Microbiol. Mol. Biol. Rev.* 62: 1-34. These carrier proteins are characterized by the presence of 12 putative transmembrane ("TM") segments. These may have evolved by duplication of an ancestral structure consisting of six transmembrane domains, as suggested by the presence of repeated structures in both halves of the molecule. These are GR(R/K) amino

acid motifs present between TM2 and TM3 and between TM8 and TM9 and the $EX_6(R/K)$ amino acid motifs present between TM4 and TM5 and between TM10 and TM11.

Within the mammalian glucose transporters, additional motifs have been characterized that are essential for transporter function. For instance, site-directed mutagenesis studies have suggested that a QLS motif present in TM7 of the high affinity glucose transporters GLUT1, 3, and 4 is important in defining binding affinity and selection of the incoming hexose. See, *e.g.*, Seatter *et al.* 1998 *Biochemistry* 37: 1322-1326. Immediately following this motif is a conserved pair of glutamine residues. The first one, Glu^{282} of GLUT1, is required for binding of the glucose competitive inhibitor ATB-BMPA on the exofacial glucose binding site. See, *e.g.*, Hashiramoto *et al.* 1992 *J. Biol. Chem.* 267: 17502-17507. Importantly also, the intracellularly located tryptophan residues 388 and 412 of GLUT1, which are conserved in GLUT2-4, participate in the conformational changes required for transport activity and for binding the competitive inhibitor cytochalasin B. See, *e.g.*, Inukai *et al.* 1994 *Biochem. J.* 302: 355-361; Garcia *et al.* 1992 *J. Biol. Chem.* 267: 7770-7776; Saravolac and Holman, 1997 In: FACILITATIVE GLUCOSE TRANSPORTERS, pp. 39-66, R. G. Landes and Co., Georgetown, TX.

The invention is based in part on the discovery of novel GLUTX nucleic acid sequences encoding polypeptides similar to the facilitative glucose transporters GLUT1-5 (Swiss-Prot accession numbers P11166, P11168, P11169, P14672, and P22732). The novel nucleic acids described herein and their encoded polypeptides are referred to collectively as "GLUTX".

The invention describes the cloning and functional characterization of novel glucose transporter proteins, including at least GLUTX1, GLUTX2 and GLUTX3, identified by data base homology searches using sequences of the known GLUTs. The GLUTX proteins share the same overall structure as the GLUTs and contains key glucose transporter sequence motifs. However, the GLUTXs have some distinctive structural features. In addition, GLUTXs have a higher homology to plant and bacterial sequences than to the mammalian GLUTs, and thus describe a novel family of mammalian facilitated glucose transporters.

GLUTX1

Included in the invention is GLUTX1, a novel glucose transporter-related polypeptide, and nucleic acids encoding the GLUTX1 polypeptide. Antibodies that bind specifically to GLUTX1 polypeptides, or fragments thereof, are also included in the invention. The invention further includes fragments, homologs, analogs, and derivatives of GLUTX1 nucleic acids, polypeptides, and antibodies. Homologs and analogs of GLUTX1 nucleic acids and proteins include at least human, rat and mouse variants described below and in GenBank™/EMBL

Data Bank accession numbers: NM_014580, NP_055395, AJ245937 and CAB75702 (human), AJ245935 and CAB75729 (rat), and AJ245936 and CAB75719 (mouse). See, Ibberson *et al.*, 2000 *J. Biol. Chem.* 275: 4607-4612.

Similarly to GLUTs1-5, GLUTX1 contains 12 putative membrane spanning domains, shown in FIG. 1, with a large cytoplasmic loop between TM 6 and TM7 but a short first exoplasmic loop which does not contain a *N*-glycosylation site. However, an *N*-glycosylation site is present in an exoplasmic loop between TM9 and TM10. This loop shows no significant homology with the other mammalian glucose transporter proteins. GLUTX1 protein sequence shows the highest degree of homology with nonmammalian sugar transporters. Identity is 36% with a putative sugar transporter from sugar beet, termed integral membrane protein (GenBank accession number AAB53155). See, *e.g.*, Chiou and Bush 1996 *Plant Physiol.* 110: 511-520. In addition, identity is 35% with *Escherichia coli* galactose-proton symport (GALP), 32% with *Lactobacillus brevis* xylose proton symport (XYLT), and between 29 and 32% with the other mammalian glucose transporters. Importantly, sequences that have been reported to be critical for the glucose transport function are present in GLUTX1 at conserved locations. These include the two repeated sequence motifs GR(R/K) and EX_6(R/K) and two tryptophan residues, Trp^{395} and Trp^{419}, corresponding to Trp^{388} and Trp^{412} of GLUT1. Interestingly, a dileucine motif is found in the amino-terminal cytoplasmic domain of the protein. Human, rat and mouse GLUTX1 sequences have been determined and are shown in Tables 3A, 3B and 3C, respectively.

Immunofluorescence and microscopic analyses were used to detect GLUTX1 and mutant GLUTX1(LL-AA) in HEK293T cells using the GLUTX1(203-257) antibody (see EXAMPLES). Secondary antibody was conjugated to CY3. The data indicate that the intracellular location of GLUTX1 depends on the presence of the dileucine internalization motif. Using extrapolation of the similarities between GLUTX family members, as defined in the EXAMPLES, one skilled in the arts would understand that the dileucine repeat may have similar or identical function in GLUTX2 and GLUTX3. Alternative embodiments of the invention include alternative sequences of any one of the GLUTX polypeptides of the invention, including GLUTX1, GLUTX2 or GLUTX3, wherein the dileucine repeat is changed to alternative amino acids, either as a repeat of a single type of amino acid, *e.g.*, dialanine, or as two separate but adjacent amino acids, *e.g.*, alanine serine, valine methionine, and the like. Substituted amino acids may be chosen from either the "strong" or "weak" groups of conserved amino acid residues, as defined below and in Example 1, or may be nonconserved. The dileucine motif is present at amino acids 12-13 of SEQ ID NO:2, amino

acids 12-13 of SEQ ID NO:4, amino acids 12-13 of SEQ ID NO:6, amino acids 22-23 of SEQ ID NO:8, amino acids 20-21 of SEQ ID NO:10, amino acids 5-6 of SEQ ID NO:12, and amino acids 5-6 of SEQ ID NO:14.

A DNA sequence of a human GLUTX1 gene (2217 nucleotides; SEQ ID NO:1), and its encoded amino acid sequence (SEQ ID NO:2), are shown in Table 3A. The translated protein is encoded from nucleotide 348 to 1778. The human GLUTX1 protein product (SEQ ID NO:2) is 477 amino acids in length. The predicted molecular weight of the human GLUTX1 polypeptide is 50858.1 Da.

A DNA sequence of a rat GLUTX1 gene (2087 nucleotides; SEQ ID NO:3), and its encoded amino acid sequence (SEQ ID NO:4), are shown in Table 3B. The translated protein is encoded from nucleotide 30 to 1463. The rat GLUTX1 protein product (SEQ ID NO:4) is 478 amino acids in length. The predicted molecular weight of the rat GLUTX1 polypeptide is 51459.0 Da.

A DNA sequence of a mouse GLUTX1 gene (2072 nucleotides; SEQ ID NO:5), and its encoded amino acid sequence (SEQ ID NO:6), are shown in Table 3C. The translated protein is encoded from nucleotide 21 to 1451. The mouse GLUTX1 protein product (SEQ ID NO:6) is 477 amino acids in length. The predicted molecular weight of the mouse GLUTX1 polypeptide is 51578.0 Da.

Further analyses of the GLUTX1 sequences are shown in the EXAMPLES section.

Mouse and rat GLUTX1 are most similar, showing 91% identity at the nucleotide and 94% identity at the amino acid sequence level; human GLUTX1 is more divergent, showing 85% identity with both the nucleotide and the protein sequence of rat and mouse GLUTX1 (FIG. 2).

Sequence comparison indicated that rat GLUTX1 was 29-32% identical to the GLUTs, whereas the GLUTs are between 40 and 70% identical to each other. Highest sequence identity (36%) was with a putative sugar transporter (integral membrane protein) cloned from sugar beet and with bacterial galactose/and xylose/proton cotransporters (35%). See, *e.g.*, Chiou and Bush 1996 *Plant Physiol.* 110: 511-520. Four small regions of GLUTX1 (45-49, 70-74, 181-188, and 398-403) show almost perfect conservation with integral membrane protein sequences that are only very distantly related to sequences at the same locations in GLUTs (indicated in FIG. 1). These regions imply differences in evolutionary origin and/or differences in structural requirements for GLUTX1 compared with the GLUTs.

Interestingly, one unique feature of GLUTX1 is the long exoplasmic loop present between TM9 and TM10 that contains the only *N*-glycosylation site of the molecule. This

sequence has no counterpart in the GLUTs or in the related plant or bacterial transporters mentioned above. However, a counterpart to this sequence does exist in GLUTX2 and GLUTX3, and GLUTX2 and GLUTX3 are presumed to contain similar domain structures as GLUTX1, as shown in FIG. 3 and the EXAMPLES. By using Smith-Waterman sequence similarity search using rat GLUTX1, we found that this sequence contains a stretch (amino acids 341-362) that is similar (54% identical over 22 residues) to a segment (amino acids 333-354) of the *msmF* multiple sugar receptor/transporter of *S. mutans*. See, *e.g.*, Smith *et al.* 1985 *Nucleic Acids Res.* 13: 645-656. The *msm*F protein has six putative transmembrane domains and forms part of a system responsible for the uptake and metabolism of multiple sugars and is closely related to the maltose transport system of *E. coli*. See, *e.g.*, Russell *et al.* 1992 *J. Biol. Chem.* 267: 4631-4637. This similarity is at present of unknown functional importance but may point to a structure that participates in sugar binding.

Chimeric and mutant glucose transporters have been widely used for the study of the structure/function of the GLUTs. See, *e.g.*, review in Saravolac and Holman 1997 In: FACILITATIVE GLUCOSE TRANSPORTERS , pp. 39-66, R. G. Landes and Co., Georgetown, TX. A recent study has identified the QLS sequence in transmembrane domain 7 of GLUT1,3,4 as a motif critical for hexose selection at the exofacial binding site. Replacing this sequence with that of human GLUT2 led to fructose, in addition to glucose, transport activity of the mutated transporter GLUTX1 has MVF at this position instead of QLS. See, *e.g.*, Seatter *et al.* 1998 *Biochemistry* 37: 1322-1326. This suggests that it may transport other hexoses beside glucose. Our oocyte uptake experiments indeed show that both fructose and galactose can compete for 2-DOG transport.

One distinguishing feature of GLUTX1 as compared with the GLUTs is its relatively short carboxyl-terminal cytoplasmic tail, which has a predicted length of 20 amino acids as compared to 42-45 amino acids for GLUTs 1-5. Deletion studies of GLUT1 have suggested that its carboxyl-terminal tail was important for allowing conformational changes that accompany glucose transport. Removing 37 amino acids of the GLUT1 tail suppressed glucose transport by locking the transporter in an inward facing conformation. See, *e.g.*, Oka *et al.* 1990 *Nature* 345: 550-553. However, shorter truncations, up to 24 amino acids (leaving an 18-amino acid-long carboxyl-terminal tail), did not affect on transport activity or cytochalasin B binding. See, *e.g.*, Saravolac and Holman 1997 In: FACILITATIVE GLUCOSE TRANSPORTERS , pp. 39-66, R. G. Landes and Co., Georgetown, TX. Therefore, by analogy, the short cytoplasmic tail of GLUTX1 should still permit normal transport activity. This was indeed confirmed by oocyte expression studies, see the EXAMPLES. These functional experiments

were, however, successful only after mutation of the dileucine motif present in the amino-terminal tail of the molecule. Transport was not inhibited by an excess of L-glucose, suggesting stereospecificity of the transport mechanism. Transport was cytochalasin B-sensitive, which is compatible with the presence of tryptophan at positions 395 and 419, which correspond to Trp^{388} and Trp^{412} of GLUT1. Transport was also indicated for fructose and galactose as assessed by competition experiments. The K_m for 2-DOG, as determined in zero-trans experiments, was ~2.4 mM. This value is similar to that of GLUT3, which is the highest affinity transporter of the GLUT family. See, *e.g.*, Gould and Holman 1993 Biochem. J. 295: 329-341.

A possible physiological role for GLUTX1 can be suggested based on both its tissue and cellular localization. First, its intracellular location suggests that it is not involved in basal cellular glucose uptake. However, similarly to GLUT4, GLUTX1 contains a dileucine internalization motif. In GLUT4, this motif, located in the carboxyl-terminal cytoplasmic tail, functions as signal for re-endocytosis following surface expression, which occurs either as part of a basal recycling process or following stimulation by insulin. See, *e.g.*, Verhey and Birnbaum 1994 *J. Biol. Chem.* 269: 2353-2356; Corvera *et al.* 1994 *J. Cell Biol.* 126: 979-989. In GLUTX1, the dileucine motif appears essential for maintaining the intracellular localization of the transporter as assessed both in transfected HEK293T cells and in neuroendocrine cells[2] and as inferred from *Xenopus* oocytes functional studies. This therefore suggests that in its normal life cycle, GLUTX1 recycles between an intracellular site and the plasma membrane. Therefore, one possible role for this transporter may be to increase cellular glucose uptake in response to certain stimuli. Those could be hormones such as insulin or neurotransmitters but also other factors such as stresses induced by, for instance, hypoxia or hypoglycemia.

The high level of GLUTX1 expression in testis suggests that this glucose transporter could function to promote glucose and fructose transport in the developing spermatocyte alongside GLUT3 and GLUT5, which are also known to be expressed in testis. See, *e.g.*, Burant *et al.* 1992 *J. Biol. Chem.* 267: 14523-14526; Younes *et al.* 1997 *Anticancer Res.* 17: 2747-2750. GLUTX1 expression is relatively high in the brain stem and hypothalamus where glucose-sensing neurons are located and that respond to hyperglycemia or hypoglycemia by increasing or decreasing their firing rates. See, *e.g.*, Oomura *et al.* 1974 *Nature* 247: 284-286; Yettefti *et al.* 1995 *J. Auton. Nerv. Syst.* 51: 191-197; Orsini *et al.* 1990 *Brain Res. Bull.* 25: 49-53. It is therefore possible that GLUTX1 plays a role in some of these glucose-sensing mechanisms or in some adaptive functions. Finally, recent studies performed with knockout mice have revealed the existence of glucose transport activity that could not be accounted for

by any of the known GLUTs. This was the case in the pancreatic cells of GLUT2-null mice, which are characterized by a loss of first phase insulin secretion but which have a preserved second phase. This remaining glucose secretory activity still depends on glucose uptake and metabolism. See, *e.g.*, Guillam *et al.* 1997 *Nat. Genet.* 17: 327-330. Glucose uptake, however, cannot be accounted for by any other known GLUTs. This was also the case in some muscles of GLUT4-null mice in which glucose uptake can still be stimulated by an insulin-dependent mechanism that does not appear to involve GLUT1-5. See, *e.g.*, Stenbit *et al.* 1997 *J. Clin. Invest.* 98: 629-634. Further localization studies, at the tissue and cellular levels, as well as evaluation of a mechanism that may induce GLUTX1 surface expression will ultimately be required to more fully elucidate the physiological role of this transporter.

GLUTX2

Included in the invention is GLUTX2, a novel glucose transporter-related polypeptide, and nucleic acids encoding the GLUTX2 polypeptide. Antibodies that bind specifically to GLUTX2 polypeptides, or fragments thereof, are included in the invention. The invention further includes fragments, homologs, analogs, and derivatives of GLUTX2 nucleic acids, polypeptides, and antibodies. Human and rat GLUTX2 sequences have been determined, and are shown in Tables 4A and 4B, respectively. Further analyses of the GLUTX2 sequences are shown in the EXAMPLES section.

A DNA sequence of a human GLUTX2 gene (2177 nucleotides; SEQ ID NO:7), and its encoded amino acid sequence (SEQ ID NO:8), are shown in Table 4A. The translated protein is encoded from nucleotide 288 to 2174. The human GLUTX2 protein product (SEQ ID NO:8) is 629 amino acids in length. The predicted molecular weight of the human GLUTX2 polypeptide is 68336.2 daltons (Da).

A DNA sequence of a rat GLUTX2 gene (2504 nucleotides; SEQ ID NO:9), and its encoded amino acid sequence (SEQ ID NO:10), are shown in Table 4B. The translated protein is encoded from nucleotide 145 to 1998. The rat GLUTX2 protein product (SEQ ID NO:10) is 618 amino acids in length. The predicted molecular weight of the rat GLUTX2 polypeptide is 66995.0 daltons.

GLUTX2 proteins are predominantly expressed in the brain. Further analyses of GLUTX2 localization, structure and homologies are shown in the EXAMPLES.

GLUTX3

Included in the invention is GLUTX3, a novel glucose transporter-related polypeptide, and nucleic acids encoding the GLUTX3 polypeptide. Antibodies that bind specifically to

GLUTX3 polypeptides, or fragments thereof, are included in the invention. The invention further includes fragments, homologs, analogs, and derivatives of GLUTX3 nucleic acids, polypeptides, and antibodies. Human and rat GLUTX3 sequences have been determined and are shown in Tables 5A and 5B, respectively.

A DNA sequence of a human GLUTX3 gene (1541 nucleotides; SEQ ID NO:11), and its encoded amino acid sequence (SEQ ID NO:12), are shown in Table 5A. The translated protein is encoded from nucleotide 11 to 1531. The human GLUTX3 protein product (SEQ ID NO:12) is 507 amino acids in length. The predicted molecular weight of the human GLUTX3 polypeptide is 54456.7 daltons.

A DNA sequence of a rat GLUTX3 gene (2011 nucleotides; SEQ ID NO:13), and its encoded amino acid sequence (SEQ ID NO:14), are shown in Table 5B. The translated protein is encoded from nucleotide 7 to 1515. The rat GLUTX3 protein product (SEQ ID NO:14) is 503 amino acids in length. The predicted molecular weight of the rat GLUTX3 polypeptide is 54899.9 daltons.

GLUTX3 proteins are predominantly expressed in the brain. Further analyses of GLUTX3 localization, structure and homologies are shown in the EXAMPLES.

GLUTX Consensus Sequence

Included in the invention are the polypeptide sequences of members of the GLUTX family of proteins, and the nucleic acids encoding those GLUTX polypeptides. Antibodies that bind specifically to GLUTX polypeptides, or fragments thereof, are included in the invention. Antibodies may be made to the various domains of the GLUTX polypeptides. Embodiments of the invention include the various TM domains, denoted as TM1 through TM 12 in Tables 3D, 4C and 5C and in FIG. 1. Additional GLUTX domains of the invention include the N-ter, C-ter, and loop regions between the various TM regions described therein. Further embodiments of invention include domains with high conservation between members of the GLUTX protein family, including those domains underlined in Tables 6A, 6B and 6D, and those stretches of residues shown in FIG. 3 with black or gray shading. Domains with high conservation between GLUTX polypeptides and the related GLUT family members are shown as black or gray shaded residues in FIG. 1. The nucleic acid, polypeptide, or both, sequences of the exoplasmic loop between TM9 and TM10 may be another targeted GLUTX domain.

Antibodies may be made to any one or more of these GLUTX protein domains. Antibodies specific to an individual GLUTX polypeptide may be made to non-consensus sequences. Antibodies with broader recognition, *i.e.*, to more than one member of the GLUTX polypeptide family, may be made against a consensus GLUTX specific sequence. Antibodies

made to consensus shaded residues shown in FIG. 1 may have even broader recognition to both the GLUTX and GLUT polypeptides. Antibodies may be made as described below.

Embodiments of the invention include GLUTX consensus nucleic acids and polypeptides encoded thereby, as well as fragments, homologs and analogs of said GLUTX consensus nucleotids and polypeptides. Antibodies to these consensus sequences, and fragments, homologs and analogs thereof, are also included in the invention, as described above. A human GLUTX polypeptide consensus sequence (SEQ ID NO:18) is shown in Table 6A. The human GLUTX consensus polypeptide sequence was derived from the Majority sequence (SEQ ID NO:21) from the human GLUTX alignment shown in Table 6E, and is further discussed in the EXAMPLES section. A ClustalX alignment of the rat GLUTX polypeptides is shown in FIG. 2. A rat GLUTX consensus sequence (SEQ ID NO:19) derived from the FIG. 3 alignment is shown in the Examples in Table 6B. An alternative alignment to FIG. 3, centered around the putative glycosylated N residue of GLUTX1-3, is shown in Table 6C, and the alternative rat GLUTX consensus sequence (SEQ ID NO:20) derived from the alternative alignment of Table 6C is shown in Table 6D. Finally, ClustalW alignment and a Majority sequence for rat GLUTX (SEQ ID NO:21) are shown in Table 6E. Predicted consensus sequences that appear specific to the GLUTX family of polypeptides are indicated by underlines.

Alternative embodiments of the invention include nucleic acids that encode these GLUTX consensus polypeptide sequence, which may be derived by a person skilled in the arts from the genetic code specifying codon designations for the various amino acid residues.

Further analyses of the GLUTX consensus sequences are shown in the EXAMPLES section.

The invention also includes regulartory sequences of GLUTX nucleic acids. Regulatory sequences include the 5' untranslated region (UTR), 3' UTR, and promoter and enhances elements, described below, of any GLUTX gene disclosed herein. The specific 5' UTRs and 3' UTRs of any of the individual GLUTX sequences may be labeled by any method known to one skilled in the art, *e.g.*, radiolabeling or fluorescence labeling as discussed below, and used to probe nucleic acid sequences, *e.g.*, genomic sequences, to identify and isolate flanking regulatory regions for the specific GLUTX gene being probed. Table 1 below summarizes the disclosed the 5' UTR, coding region (CDS), length in amino acids of the predicted GLUTX polypeptide, and 3' UTR for the various GLUTX genes disclosed herein.

TABLE 1

GLUTX	SEQ ID NO:	5'UTR	CDS	Amino Acids	3'UTR
hX1	1	1-347	348-1778	477	1779-2217
hX2	7	1-287	288-2174	629	2175-2177
hX3	11	1-10	11-1531	507	1532-1541
mX1	5	1-20	21-1451	477	4152-2072
rX1	3	1-29	30-1463	478	1464-2087
rX2	9	1-144	145-1998	618	1999-2304
rX3	13	1-6	7-1313	503	1316-2011

GLUTX Nucleic Acids

TABLE 2 below summarizes the disclosed GLUTX nucleic acid sequences, encoded polypeptides, and the corresponding sequence identifier numbers (SEQ ID NOs) for the various disclosed sequences and clones containing these nucleic acids and polypeptides.

TABLE 2: Summary Of Nucleic Acids And Proteins Of The Invention

GLUTX	SPECIES	TABLE	NUCLEIC ACID SEQ ID NOs	POLYPEPTIDE SEQ ID NOs
GLUTX1	human	3A	SEQ ID NO:1	SEQ ID NO:2
	rat	3B	SEQ ID NO:3	SEQ ID NO:4
	mouse	3C	SEQ ID NO:5	SEQ ID NO:6
GLUTX2	human	4A	SEQ ID NO:7	SEQ ID NO:8
	rat	4B	SEQ ID NO 9	SEQ ID NO:10
GLUTX3	human	5A	SEQ ID NO:11	SEQ ID NO:12
	rat	5B	SEQ ID NO:13	SEQ ID NO:14
GLUTX#	X1 majority	3D		SEQ ID NO:15
	X2 majority	4C		SEQ ID NO:16
	X3 majority	5C		SEQ ID NO:17
GLUTX1-3	human consensus	6A		SEQ ID NO:18
	rat consensus	6B		SEQ ID NO:19
	alt rat consensus	6D		SEQ ID NO:20
GLUTX1-3	human majority	6E		SEQ ID NO:21
	rat majority	6F		SEQ ID NO:22

The novel nucleic acids of the invention include those that encode a GLUTX or GLUTX-like protein. Among these nucleic acids is the nucleic acid whose sequence is provided in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13, or a fragment, derivative or homolog thereof. Additionally, the invention includes mutant or variant nucleic acids of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13, or a fragment, derivative or homolog thereof, any of whose bases may be changed from the corresponding base shown in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13, while still encoding a protein that maintains its GLUTX -like activities and physiological functions. The invention further includes the complement of the nucleic acid sequence of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13, including fragments, derivatives, analogs and homolog thereof. Examples of the

complementary strand of portions of GLUTX may be determined by Watson-Crick base pairing, wherein adenine (A) pairs with thymidine (T) and guanine (G) pairs with cytosine (C), which is known to anyone skill in the art. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications.

One aspect of the invention pertains to isolated nucleic acid molecules that encode GLUTX proteins or biologically active portions thereof. Also included are nucleic acid fragments sufficient for use as hybridization probes to identify GLUTX-encoding nucleic acids (*e.g.*, GLUTX mRNA) and fragments for use as polymerase chain reaction (PCR) primers for the amplification or mutation of GLUTX nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

"Probes" refer to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 100 nt, or as many as about, *e.g.*, 6,000 nt, depending on use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are usually obtained from a natural or recombinant source, are highly specific and much slower to hybridize than oligomers. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

An "isolated" nucleic acid molecule is one that is separated from other nucleic acid molecules that are present in the natural source of the nucleic acid. Examples of isolated nucleic acid molecules include, but are not limited to, recombinant DNA molecules contained in a vector, recombinant DNA molecules maintained in a heterologous host cell, partially or substantially purified nucleic acid molecules, and synthetic DNA or RNA molecules. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated GLUTX nucleic acid molecule can contain less than about 50 kb, 25 kb, 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of

other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 or a complement of any of this nucleotide sequence, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 as a hybridization probe, GLUTX nucleic acid sequences can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook *et al.*, eds., MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, eds., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to GLUTX nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nt in length, preferably about 15 nt to 30 nt in length. In one embodiment, an oligonucleotide comprising a nucleic acid molecule less than 100 nt in length would further comprise at lease 6 contiguous nucleotides of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 or a complement thereof. Oligonucleotides may be chemically synthesized and may be used as probes.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13. In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 or a portion of this nucleotide sequence. A nucleic acid molecule that is complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 5,

7, 9, 11 or 13 is one that is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 that it can hydrogen bond with little or no mismatches to the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 , thereby forming a stable duplex.

As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term "binding" means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, Von der Waals, hydrophobic interactions, etc. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 , *e.g.*, a fragment that can be used as a probe or primer, or a fragment encoding a biologically active portion of GLUTX. "Fragments" provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. "Derivatives" are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. "Analogs" are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or analogs of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 70%, 80%, 85%, 90%, 95%, 98%, or even 99% identity (with a preferred identity of 80-99%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the

alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below. An exemplary program is the Gap program (Wisconsin Sequence Analysis Package, Version 8 for UNIX, Genetics Computer Group, University Research Park, Madison, WI) using the default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2: 482-489, which is incorporated herein by reference in its entirety).

A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of GLUTX polypeptide. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the present invention, homologous nucleotide sequences include nucleotide sequences encoding for a GLUTX polypeptide of species other than humans, including, but not limited to, mammals, and thus can include, *e.g.*, mouse, rat, rabbit, dog, cat cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the nucleotide sequence encoding human GLUTX protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21, as well as a polypeptide having GLUTX activity. Biological activities of the GLUTX proteins are described below. A homologous amino acid sequence does not encode the amino acid sequence of a human GLUTX polypeptide.

The nucleotide sequence determined from the cloning of the human GLUTX gene allows for the generation of probes and primers designed for use in identifying and/or cloning GLUTX homologues in other cell types, *e.g.*, from other tissues, as well as GLUTX homologues from other mammals. The probe/primer typically comprises a substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 or more consecutive sense strand nucleotide sequence of SEQ ID NO:1,

3, 5, 7, 9, 11 or 13; or an anti-sense strand nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13; or of a naturally occurring mutant of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13.

Probes based on the human GLUTX nucleotide sequence can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a GLUTX protein, such as by measuring a level of a GLUTX-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting GLUTX mRNA levels or determining whether a genomic GLUTX gene has been mutated or deleted.

A "polypeptide having a biologically active portion of GLUTX" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically active portion of GLUTX" can be prepared by isolating a portion of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 that encodes a polypeptide having a GLUTX biological activity (biological activities of the GLUTX proteins are described below), expressing the encoded portion of GLUTX protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of GLUTX. For example, a nucleic acid fragment encoding a biologically active portion of GLUTX can optionally include an ATP-binding domain. In another embodiment, a nucleic acid fragment encoding a biologically active portion of GLUTX includes one or more regions.

GLUTX variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences shown in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 due to degeneracy of the genetic code. These nucleic acids thus encode the same GLUTX protein as that encoded by the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21.

In addition to the human GLUTX nucleotide sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of GLUTX may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the GLUTX gene may exist among individuals within a population due to natural allelic variation. As used

herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a GLUTX protein, preferably a mammalian GLUTX protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the GLUTX gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in GLUTX that are the result of natural allelic variation and that do not alter the functional activity of GLUTX are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding GLUTX proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the GLUTX cDNAs of the invention can be isolated based on their homology to the human GLUTX nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. For example, a soluble human GLUTX cDNA can be isolated based on its homology to human membrane-bound GLUTX. Likewise, a membrane-bound human GLUTX cDNA can be isolated based on its homology to soluble human GLUTX.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500 or 750 nucleotides in length. In another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

Homologs (*i.e.*, nucleic acids encoding GLUTX proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5°C lower than the

thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at Tm, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (*e.g.*, 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions is hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C. This hybridization is followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 corresponds to a naturally occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 , or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well known in the art. See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 or fragments, analogs

or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations). See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981, *Proc Natl Acad Sci USA 78*: 6789-6792.

Conservative mutations

In addition to naturally-occurring allelic variants of the GLUTX sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13, thereby leading to changes in the amino acid sequence of the encoded GLUTX protein, without altering the functional ability of the GLUTX protein. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of GLUTX without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the GLUTX proteins of the present invention, are predicted to be particularly unamenable to alteration.

In addition, amino acid residues that are conserved among GLUTX members, as indicated by the alignments presented as TABLES 6A through 6E and in FIGS. 1-3, are predicted to be less amenable to alteration. For example, GLUTX proteins of the present invention can contain at least one domain that is a typically conserved region in GLUTX members, *i.e.*, GLUTX1, GLUTX2, or GLUTX3 proteins, and GLUTX homologs. As such, these conserved domains are not likely to be amenable to mutation. Other amino acid residues, however, (*e.g.*, those that are not conserved or only semi-conserved among members of the GLUTX proteins) may not be as essential for activity and thus are more likely to be amenable to alteration.

Another aspect of the invention pertains to nucleic acid molecules encoding GLUTX proteins that contain changes in amino acid residues that are not essential for activity. Such GLUTX proteins differ in amino acid sequence from SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16,

17, 18, 19, 20 or 21, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 75% homologous to the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21. Preferably, the protein encoded by the nucleic acid is at least about 80% homologous to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21, more preferably at least about 90%, 95%, 98%, and most preferably at least about 99% homologous to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21.

An isolated nucleic acid molecule encoding a GLUTX protein homologous to the protein of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in GLUTX is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a GLUTX coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for GLUTX biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13, the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

In one embodiment, a mutant GLUTX protein can be assayed for (*i*) the ability to form protein:protein interactions with other GLUTX proteins, other cell-surface proteins, or biologically active portions thereof, (*ii*) complex formation between a mutant GLUTX protein

and a GLUTX receptor; (*iii*) the ability of a mutant GLUTX protein to bind to an intracellular target protein or biologically active portion thereof; (*e.g.*, avidin proteins); (*iv*) the ability to bind BRA protein; or (*v*) the ability to specifically bind an anti-GLUTX protein antibody.

Antisense

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire GLUTX coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of a GLUTX protein of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21, or antisense nucleic acids complementary to a GLUTX nucleic acid sequence of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding GLUTX. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the protein coding region of human GLUTX corresponds to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding GLUTX. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding GLUTX disclosed herein (*e.g.*, SEQ ID NO:1, 3, 5, 7, 9, 11 or 13), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of GLUTX mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of GLUTX mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of GLUTX mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis

or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used.

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-thiocytosine, 5-methyl-2-thiouracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, N6-adenine, (acp3)w, 2,6-diaminopurine and 3-(3-amino-3-N-2-carboxypropyl) uracil. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a GLUTX protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell

surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier *et al.* 1987 *Nucleic Acids Res* 15: 6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* 1987 *Nucleic Acids Res* 15: 6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* 1987 *FEBS Lett* 215: 327-330).

Ribozymes and PNA moieties

Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (see, *e.g.*, Haselhoff and Gerlach 1988 *Nature* 334:585-591)) can be used to catalytically cleave GLUTX mRNA transcripts to thereby inhibit translation of GLUTX mRNA. A ribozyme having specificity for a GLUTX-encoding nucleic acid can be designed based upon the nucleotide sequence of a GLUTX DNA disclosed herein (*i.e.*, SEQ ID NO:1, 3, 5, 7, 9, 11 or 13). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a GLUTX-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Pat. No. 4,987,071; and Cech *et al.* U.S. Pat. No. 5,116,742. Alternatively, GLUTX mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.* 1993 *Science* 261:1411-1418.

Alternatively, GLUTX gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the GLUTX (*e.g.*, the GLUTX promoter and/or enhancers) to form triple helical structures that prevent transcription of the GLUTX

gene in target cells. See generally, Helene 1991 *Anticancer Drug Des.* 6: 569-84; Helene. *et al.* 1992 *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher 1992 *Bioassays* 14: 807-15.

In various embodiments, the nucleic acids of GLUTX can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids. See, *e.g.*, Hyrup *et al.* 1996 *Bioorg Med Chem* 4: 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols. See, *e.g.*, Hyrup *et al.* (1996) above; Perry-O'Keefe *et al.* 1996 *PNAS* 93: 14670-675.

PNAs of GLUTX can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs of GLUTX can also be used, *e.g.*, in the analysis of single base pair mutations in a gene by, *e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S1 nucleases; or as probes or primers for DNA sequence and hybridization. See, *e.g.*, Hyrup *et al.* 1996, above; Perry-O'Keefe *et al.* 1996, above).

In another embodiment, PNAs of GLUTX can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of GLUTX can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, *e.g.*, RNase H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation. See, *e.g.*, Hyrup *et al.* 1996, above. The PNA-DNA chimeras can be synthesized. See, *e.g.*, Hyrup *et al.* 1996, above, and Finn *et al.* 1996 *Nucl Acids Res* 24: 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl) amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA. See, *e.g.*, Mag *et al.* 1989 *Nucl Acid Res* 17:

5973-88. PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment. See, *e.g.*, Finn *et al.* 1996, above. Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. See, *e.g.*, Petersen *et al.* 1975 *Bioorg Med Chem Lett* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre *et al.*, 1987, Proc. *Natl. Acad. Sci.* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (see, *e.g.*, Krol *et al.*, 1988, *BioTechniques* 6:958-976) or intercalating agents (see, *e.g.*, Zon, 1988, *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, etc.

GLUTX polypeptides

The novel protein of the invention includes the GLUTX-like protein whose sequence is provided in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21. The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21 while still encoding a protein that maintains its GLUTX-like activities and physiological functions, or a functional fragment thereof. In the mutant or variant protein, up to 20% or more of the residues may be so changed.

In general, an GLUTX -like variant that preserves GLUTX-like function includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further include the possibility of inserting an additional residue or residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative substitution as defined above. In other embodiments, the substitutions are at non-conserved positions, as described by dashes (-) or lower case letters in the Tables in the EXAMPLES.

One aspect of the invention pertains to isolated GLUTX proteins, and biologically active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-GLUTX antibodies. In

one embodiment, native GLUTX proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, GLUTX proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a GLUTX protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the GLUTX protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of GLUTX protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of GLUTX protein having less than about 30% (by dry weight) of non-GLUTX protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-GLUTX protein, still more preferably less than about 10% of non-GLUTX protein, and most preferably less than about 5% non-GLUTX protein. When the GLUTX protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of GLUTX protein in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of GLUTX protein having less than about 30% (by dry weight) of chemical precursors or non-GLUTX chemicals, more preferably less than about 20% chemical precursors or non-GLUTX chemicals, still more preferably less than about 10% chemical precursors or non-GLUTX chemicals, and most preferably less than about 5% chemical precursors or non-GLUTX chemicals.

Biologically active portions of a GLUTX protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the GLUTX protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21 that include fewer amino acids than the full length GLUTX proteins, and exhibit at least one activity of a GLUTX protein. Typically, biologically active portions

comprise a domain or motif with at least one activity of the GLUTX protein. A biologically active portion of a GLUTX protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

A biologically active portion of a GLUTX protein of the present invention may contain at least one of the above-identified domains conserved between the GLUTX proteins. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native GLUTX protein.

In an embodiment, the GLUTX protein has an amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21. In other embodiments, the GLUTX protein is substantially homologous to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21 and retains the functional activity of the protein of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail below. Accordingly, in another embodiment, the GLUTX protein is a protein that comprises an amino acid sequence at least about 45% homologous to the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21 and retains the functional activity of the GLUTX proteins of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21.

Determining homology between two or more sequences

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in either of the sequences being compared for optimal alignment between the sequences). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. See, *Needleman and Wunsch* 1970 *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a

degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NO:1, 3, 5, 7, 9, 11 or 13.

The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region. The term "percentage of positive residues" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical and conservative amino acid substitutions, as defined above, occur in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of positive residues.

Chimeric and fusion proteins

The invention also provides GLUTX chimeric or fusion proteins. As used herein, a GLUTX "chimeric protein" or "fusion protein" comprises a GLUTX polypeptide operatively linked to a non-GLUTX polypeptide. A "GLUTX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to GLUTX, whereas a "non-GLUTX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the GLUTX protein, *e.g.*, a protein that is different from the GLUTX protein and that is derived from the same or a different organism. Within a GLUTX fusion protein the GLUTX polypeptide can correspond to all or a portion of a GLUTX protein. In one embodiment, a GLUTX fusion protein comprises at least one biologically active portion of a GLUTX protein. In another embodiment, a GLUTX fusion protein comprises at least two biologically active portions of a GLUTX protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the GLUTX polypeptide and the non-GLUTX

polypeptide are fused in-frame to each other. The non-GLUTX polypeptide can be fused to the N-terminus or C-terminus of the GLUTX polypeptide.

For example, in one embodiment a GLUTX fusion protein comprises a GLUTX polypeptide operably linked to the extracellular domain of a second protein. Such fusion proteins can be further utilized in screening assays for compounds that modulate GLUTX activity (such assays are described in detail below).

In another embodiment, the fusion protein is a GST-GLUTX fusion protein in which the GLUTX sequences are fused to the C-terminus of the GST (*i.e.*, glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant GLUTX.

In yet another embodiment, the fusion protein is a GLUTX protein containing a heterologous signal sequence at its N-terminus. For example, the native GLUTX signal sequence (*i.e.*, amino acids 1 to 20 of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21) can be removed and replaced with a signal sequence from another protein. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of GLUTX can be increased through use of a heterologous signal sequence.

In another embodiment, the fusion protein is a GLUTX-immunoglobulin fusion protein in which the GLUTX sequences comprising one or more domains are fused to sequences derived from a member of the immunoglobulin protein family. The GLUTX-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a GLUTX ligand and a GLUTX protein on the surface of a cell, to thereby suppress GLUTX-mediated signal transduction *in vivo*. In one nonlimiting example, a contemplated GLUTX ligand of the invention is the GLUTX receptor. The GLUTX-immunoglobulin fusion proteins can be used to affect the bioavailability of a GLUTX cognate ligand. Inhibition of the GLUTX ligand/GLUTX interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as modulating (*e.g.*, promoting or inhibiting) cell survival. Moreover, the GLUTX-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-GLUTX antibodies in a subject, to purify GLUTX ligands, and in screening assays to identify molecules that inhibit the interaction of GLUTX with a GLUTX ligand.

A GLUTX chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction

enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Ausubel *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A GLUTX-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the GLUTX protein.

GLUTX agonists and antagonists

The present invention also pertains to variants of the GLUTX proteins that function as either GLUTX agonists (mimetics) or as GLUTX antagonists. Variants of the GLUTX protein can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of the GLUTX protein. An agonist of the GLUTX protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the GLUTX protein. An antagonist of the GLUTX protein can inhibit one or more of the activities of the naturally occurring form of the GLUTX protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the GLUTX protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the GLUTX proteins.

Variants of the GLUTX protein that function as either GLUTX agonists (mimetics) or as GLUTX antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of the GLUTX protein for GLUTX protein agonist or antagonist activity. In one embodiment, a variegated library of GLUTX variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of GLUTX variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential GLUTX sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of GLUTX sequences therein. There are a variety of methods which can be used to produce libraries of potential

GLUTX variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential GLUTX sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu Rev Biochem* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucl Acid Res* 11:477.

Polypeptide libraries

In addition, libraries of fragments of the GLUTX protein coding sequence can be used to generate a variegated population of GLUTX fragments for screening and subsequent selection of variants of a GLUTX protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a GLUTX coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal and internal fragments of various sizes of the GLUTX protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of GLUTX proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recrusive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify GLUTX variants (Arkin and Yourvan 1992 PNAS 89:7811-7815; Delgrave *et al.* 1993 Protein Engineering 6:327-331).

Anti-GLUTX Antibodies

The invention further encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to any of the proteins of the invention.

An isolated GLUTX protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind GLUTX using standard techniques for polyclonal and monoclonal antibody preparation. Full-length GLUTX protein can be used. Alternatively, the invention provides antigenic peptide fragments of GLUTX for use as immunogens. The antigenic peptide of GLUTX comprises at least 4 amino acid residues of the amino acid sequence shown in SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21. The antigenic peptide encompasses an epitope of GLUTX such that an antibody raised against the peptide forms a specific immune complex with GLUTX. The antigenic peptide may comprise at least 6 aa residues, at least 8 aa residues, at least 10 aa residues, at least 15 aa residues, at least 20 aa residues, or at least 30 aa residues. In one embodiment of the invention, the antigenic peptide comprises a polypeptide comprising at least 6 contiguous amino acids of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21.

In an embodiment of the invention, epitopes encompassed by the antigenic peptide are regions of GLUTX that are located on the surface of the protein, *e.g.*, hydrophilic regions. A hydrophobicity analysis of the human GLUTX protein sequence would indicate that the regions between the TM domains are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting antibody production. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, *e.g.*, Hopp and Woods, 1981, Proc. Nat. Acad. Sci. USA 78: 3824-3828; Kyte and Doolittle 1982, J. Mol. Biol. 157: 105-142, each incorporated herein by reference in their entirety.

As disclosed herein, GLUTX protein sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen, such as GLUTX. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab} and $F_{(ab')2}$ fragments, and an F_{ab} expression library. In a specific embodiment, antibodies to human GLUTX proteins are disclosed. Various procedures known within the art

may be used for the production of polyclonal or monoclonal antibodies to a GLUTX protein sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 15,.16, 17, 18, 19, 20 or 21, or a derivative, fragment, analog or homolog thereof. Some of these proteins are discussed below.

For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, or a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, recombinantly expressed GLUTX protein or a chemically synthesized GLUTX polypeptide. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), human adjuvants such as *Bacille Calmette-Guerin* and *Corynebacterium parvum*, or similar immunostimulatory agents. If desired, the antibody molecules directed against GLUTX can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction.

The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of GLUTX. A monoclonal antibody composition thus typically displays a single binding affinity for a particular GLUTX protein with which it immunoreacts. For preparation of monoclonal antibodies directed towards a particular GLUTX protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique (see Kohler & Milstein, 1975 *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (see Kozbor, *et al.*, 1983 *Immunol Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, *et al.*, 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, *et al.*, 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (see Cole, *et al.*, 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Each of the above citations are incorporated herein by reference in their entirety.

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to a GLUTX protein (see *e.g.*, U.S. Patent No. 4,946,778). In

addition, methods can be adapted for the construction of F_{ab} expression libraries (see *e.g.*, Huse, *et al.*, 1989 *Science* 246: 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for a GLUTX protein or derivatives, fragments, analogs or homologs thereof. Non-human antibodies can be "humanized" by techniques well known in the art. See *e.g.*, U.S. Patent No. 5,225,539. Each of the above citations are incorporated herein by reference. Antibody fragments that contain the idiotypes to a GLUTX protein may be produced by techniques known in the art including, but not limited to: (*i*) an $F_{(ab')2}$ fragment produced by pepsin digestion of an antibody molecule; (*ii*) an F_{ab} fragment generated by reducing the disulfide bridges of an $F_{(ab')2}$ fragment; (*iii*) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (*iv*) F_v fragments.

Additionally, recombinant anti-GLUTX antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT International Application No. PCT/US86/02269; European Patent Application No. 184,187; European Patent Application No. 171,496; European Patent Application No. 173,494; PCT International Publication No. WO 86/01533; U.S. Pat. No. 4,816,567; European Patent Application No. 125,023; Better *et al.*(1988) *Science* 240:1041-1043; Liu *et al.* (1987) *PNAS* 84:3439-3443; Liu *et al.* (1987) *J Immunol.* 139:3521-3526; Sun *et al.* (1987) *PNAS* 84:214-218; Nishimura *et al.* (1987) *Cancer Res* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; Shaw *et al.* (1988), *J. Natl Cancer Inst* 80:1553-1559); Morrison(1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; U.S. Pat. No. 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J Immunol* 141:4053-4060. Each of the above citations are incorporated herein by reference.

In one embodiment, methods for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of a GLUTX protein is facilitated by generation of hybridomas that bind to the fragment of a GLUTX protein possessing such a domain. Antibodies that are specific for one or more domains within a GLUTX protein, *e.g.*, any one or more of the cytoplasmic or extracellular loop regions between the TM domains, specific to GLUTX family member, GLUTX1, GLUTX2 or

GLUTX3 when compared to GLUT1-5, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

Anti-GLUTX antibodies may be used in methods known within the art relating to the localization and/or quantitation of a GLUTX protein (*e.g.*, for use in measuring levels of the GLUTX protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies for GLUTX proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antibody derived binding domain, are utilized as pharmacologically-active compounds [hereinafter "Therapeutics"].

An anti-GLUTX antibody (*e.g.*, monoclonal antibody) can be used to isolate GLUTX by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-GLUTX antibody can facilitate the purification of natural GLUTX from cells and of recombinantly produced GLUTX expressed in host cells. Moreover, an anti-GLUTX antibody can be used to detect GLUTX protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the GLUTX protein. Anti-GLUTX antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

GLUTX Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding GLUTX protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA

segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, GLUTX proteins, mutant forms of GLUTX, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of GLUTX in prokaryotic or eukaryotic cells. For example, GLUTX can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (1) to increase expression of recombinant protein; (2) to increase the solubility of the recombinant protein; and (3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. See, Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the GLUTX expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerivisae* include pYepSec1 (Baldari, *et al.*, (1987) *EMBO J* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

Alternatively, GLUTX can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith *et al.* (1983) *Mol Cell Biol* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells. See, *e.g.*, Chapters 16 and 17 of Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv Immunol* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to GLUTX mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub *et al.*, "Antisense RNA as a molecular tool for genetic analysis," Reviews--Trends in Genetics, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, GLUTX protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding GLUTX or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) GLUTX protein. Accordingly, the invention further provides methods for producing GLUTX protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding GLUTX has been introduced) in a suitable medium such that GLUTX protein is produced. In another embodiment, the method further comprises isolating GLUTX from the medium or the host cell.

Transgenic animals

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which GLUTX-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous GLUTX sequences have been introduced into their genome or homologous recombinant animals in which endogenous GLUTX sequences have been altered. Such animals are useful for studying the function and/or activity of GLUTX and for identifying and/or evaluating modulators of GLUTX activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous GLUTX gene has

been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing GLUTX-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The human GLUTX DNA sequence of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of the human GLUTX gene, such as a mouse GLUTX gene, can be isolated based on hybridization to the human GLUTX cDNA (described further above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the GLUTX transgene to direct expression of GLUTX protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Pat. Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan 1986, In: MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the GLUTX transgene in its genome and/or expression of GLUTX mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding GLUTX can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a GLUTX gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the GLUTX gene. The GLUTX gene can be a human gene (*e.g.*, SEQ ID NO:1, 3, 5, 7, 9, 11 or 13), but more preferably, is a non-human homologue of a human GLUTX gene. For example, a mouse homologue of human GLUTX gene of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 can be used to construct a homologous recombination vector suitable for altering an endogenous GLUTX gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous GLUTX gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous GLUTX gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous GLUTX protein). In the homologous recombination vector, the altered portion of the GLUTX gene is flanked at its 5' and 3' ends by additional nucleic acid of the GLUTX gene to allow for homologous recombination to occur between the exogenous GLUTX gene carried by the vector and an endogenous GLUTX gene in an embryonic stem cell. The additional flanking GLUTX nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector. See *e.g.*, Thomas *et al.* (1987) *Cell* 51:503 for a description of homologous recombination vectors. The vector is introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced GLUTX gene has homologously recombined with the endogenous GLUTX gene are selected (see *e.g.*, Li *et al.* (1992) *Cell* 69:915).

The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. See *e.g.*, Bradley 1987, In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley (1991) *Curr Opin Biotechnol* 2:823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, see, *e.g.*, Lakso *et al.* (1992) *PNAS* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one

containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut *et al.* (1997) *Nature* 385:810-813. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G_0 phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

Pharmaceutical Compositions

The GLUTX nucleic acid molecules, GLUTX proteins, and anti-GLUTX antibodies (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine,

propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a GLUTX protein or anti-GLUTX antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of

the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be

obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by any of a number of routes, *e.g.*, as described in U.S. Patent Nos. 5,703,055. Delivery can thus also include, *e.g.*, intravenous injection, local administration (see U.S. Pat. No. 5,328,470) or stereotactic injection (see *e.g.*, Chen *et al.* (1994) *PNAS* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: (a) screening assays; (b) detection assays (*e.g.*, chromosomal mapping, tissue typing, forensic biology), (c) predictive medicine (*e.g.*, diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenomics); and (d) methods of treatment (*e.g.*, therapeutic and prophylactic). As described herein, in one embodiment, a GLUTX protein of the invention has the ability to bind ATP.

The isolated nucleic acid molecules of the invention can be used to express GLUTX protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect GLUTX mRNA (*e.g.*, in a biological sample) or a genetic lesion in a GLUTX gene, and to modulate GLUTX activity, as described further below. In addition, the GLUTX proteins can be used to screen drugs or compounds that modulate the GLUTX activity or expression as well as to treat disorders characterized by insufficient or excessive production of GLUTX protein, for example proliferative or differentiative disorders, or production of GLUTX protein forms that have decreased or aberrant activity compared to GLUTX wild type protein. In addition, the anti-GLUTX antibodies of the invention can be used to detect and isolate GLUTX proteins and modulate GLUTX activity.

This invention further pertains to novel agents identified by the above described screening assays and uses thereof for treatments as described herein.

Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to GLUTX proteins or have a stimulatory or inhibitory effect on, for example, GLUTX expression or GLUTX activity.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a GLUTX protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) *Anticancer Drug Des* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc Natl Acad Sci U.S.A.* 90:6909; Erb *et al.* (1994) *Proc Natl Acad Sci U.S.A.* 91:11422; Zuckermann *et al.* (1994) *J Med Chem* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew Chem Int Ed Engl* 33:2059; Carell *et al.* (1994) *Angew Chem Int Ed Engl* 33:2061; and Gallop *et al.* (1994) *J Med Chem* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), on chips (Fodor

(1993) *Nature* 364:555-556), bacteria (Ladner U.S. Pat. No. 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390; Devlin (1990) *Science* 249:404-406; Cwirla *et al.* (1990) *Proc Natl Acad Sci U.S.A.* 87:6378-6382; Felici (1991) *J Mol Biol* 222:301-310; Ladner above.).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of GLUTX protein, or a biologically active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to a GLUTX protein determined. The cell, for example, can of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the GLUTX protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the GLUTX protein or biologically active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of GLUTX protein, or a biologically active portion thereof, on the cell surface with a known compound which binds GLUTX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a GLUTX protein, wherein determining the ability of the test compound to interact with a GLUTX protein comprises determining the ability of the test compound to preferentially bind to GLUTX or a biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of GLUTX protein, or a biologically active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the GLUTX protein or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of GLUTX or a biologically active portion thereof can be accomplished, for example, by determining the ability of the GLUTX protein to bind to or interact with a GLUTX target molecule. As used herein, a "target molecule" is a molecule with which a GLUTX protein binds or interacts in nature, for example, a molecule on the surface of a cell

which expresses a GLUTX interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. A GLUTX target molecule can be a non-GLUTX molecule or a GLUTX protein or polypeptide of the present invention. In one embodiment, a GLUTX target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.*, a signal generated by binding of a compound to a membrane-bound GLUTX molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that has catalytic activity or a protein that facilitates the association of downstream signaling molecules with GLUTX.

Determining the ability of the GLUTX protein to bind to or interact with a GLUTX target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the GLUTX protein to bind to or interact with a GLUTX target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (*i.e.* intracellular Ca^{2+}, diacylglycerol, IP_3, etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a GLUTX-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the present invention is a cell-free assay comprising contacting a GLUTX protein or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to the GLUTX protein or biologically active portion thereof. Binding of the test compound to the GLUTX protein can be determined either directly or indirectly as described above. In one embodiment, the assay comprises contacting the GLUTX protein or biologically active portion thereof with a known compound which binds GLUTX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a GLUTX protein, wherein determining the ability of the test compound to interact with a GLUTX protein comprises determining the ability of the test compound to preferentially bind to GLUTX or biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-free assay comprising contacting GLUTX protein or biologically active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the GLUTX protein

or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of GLUTX can be accomplished, for example, by determining the ability of the GLUTX protein to bind to a GLUTX target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of GLUTX can be accomplished by determining the ability of the GLUTX protein further modulate a GLUTX target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as previously described.

In yet another embodiment, the cell-free assay comprises contacting the GLUTX protein or biologically active portion thereof with a known compound which binds GLUTX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a GLUTX protein, wherein determining the ability of the test compound to interact with a GLUTX protein comprises determining the ability of the GLUTX protein to preferentially bind to or modulate the activity of a GLUTX target molecule.

The cell-free assays of the present invention are amenable to use of both the soluble form or the membrane-bound form of GLUTX. In the case of cell-free assays comprising the membrane-bound form of GLUTX, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of GLUTX is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_n$, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl)dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either GLUTX or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to GLUTX, or interaction of GLUTX with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-GLUTX fusion proteins or GST-target fusion proteins can be

adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or GLUTX protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of GLUTX binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either GLUTX or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated GLUTX or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with GLUTX or target molecules, but which do not interfere with binding of the GLUTX protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or GLUTX trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the GLUTX or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the GLUTX or target molecule.

In another embodiment, modulators of GLUTX expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of GLUTX mRNA or protein in the cell is determined. The level of expression of GLUTX mRNA or protein in the presence of the candidate compound is compared to the level of expression of GLUTX mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of GLUTX expression based on this comparison. For example, when expression of GLUTX mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of GLUTX mRNA or protein expression. Alternatively, when expression of GLUTX mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of GLUTX mRNA or protein expression. The level of GLUTX

mRNA or protein expression in the cells can be determined by methods described herein for detecting GLUTX mRNA or protein.

In yet another aspect of the invention, the GLUTX proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (see, *e.g.*, U.S. Pat. No. 5,283,317; Zervos *et al.* (1993) Cell 72:223-232; Madura *et al.* (1993) J Biol Chem 268:12046-12054; Bartel *et al.* (1993) Biotechniques 14:920-924; Iwabuchi *et al.* (1993) Oncogene 8:1693-1696; and Brent WO94/10300), to identify other proteins that bind to or interact with GLUTX ("GLUTX-binding proteins" or "GLUTX-bp") and modulate GLUTX activity. Such GLUTX-binding proteins are also likely to be involved in the propagation of signals by the GLUTX proteins as, for example, upstream or downstream elements of the GLUTX pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for GLUTX is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a GLUTX-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with GLUTX.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample.

The GLUTX sequences of the present invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA

is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the present invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Pat. No. 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the GLUTX sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The GLUTX sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13, as described above, can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining GLUTX protein and/or nucleic acid expression as well as GLUTX activity, in the context of a biological sample (*e.g.*, blood, serum, cells, tissue) to thereby determine whether an individual

is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant GLUTX expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with GLUTX protein, nucleic acid expression or activity. For example, mutations in a GLUTX gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with GLUTX protein, nucleic acid expression or activity.

Another aspect of the invention provides methods for determining GLUTX protein, nucleic acid expression or GLUTX activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of GLUTX in clinical trials.

These and other agents are described in further detail in the following sections.

Diagnostic Assays

Many, if not all, homologous proteins are known in the art to have closely related or identical functions. See, *e.g.*, Lewin, "Chapter 21: Structural Genes Belong to Families" In: GENES II, 1985, John Wiley and Sons, Inc., New York. The GLUTX polypeptide have homology to the GLUT1-5 and GLUT8 proteins, which was shown previously to be specifically involved in facultative transport of hexose (see above). Therefore, it is presumed that GLUTX would also modulate transport of hexose. GLUTX may thus be particularly useful in diagnosing disorders related to aberrant hexose regulation and the like. Oligonucleotides corresponding to any one portion of the GLUTX nucleic acids of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13 may be used to detect the expression of a GLUTX-like gene. The proteins of the invention may be used to stimulate production of antibodies specifically binding the proteins. Such antibodies may be used in immunodiagnostic procedures to detect the occurrence of the protein in a sample.

An exemplary method for detecting the presence or absence of GLUTX in a biological sample involves obtaining a biological sample from a test subject and contacting the biological

sample with a compound or an agent capable of detecting GLUTX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes GLUTX protein such that the presence of GLUTX is detected in the biological sample. An agent for detecting GLUTX mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to GLUTX mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length GLUTX nucleic acid, such as the nucleic acid of SEQ ID NO:1, 3, 5, 7, 9, 11 or 13, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to GLUTX mRNA or genomic DNA, as described above. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting GLUTX protein is an antibody capable of binding to GLUTX protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect GLUTX mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of GLUTX mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of GLUTX protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of GLUTX genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of GLUTX protein include introducing into a subject a labeled anti-GLUTX antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test

subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting GLUTX protein, mRNA, or genomic DNA, such that the presence of GLUTX protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of GLUTX protein, mRNA or genomic DNA in the control sample with the presence of GLUTX protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of GLUTX in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting GLUTX protein or mRNA in a biological sample; means for determining the amount of GLUTX in the sample; and means for comparing the amount of GLUTX in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect GLUTX protein or nucleic acid.

Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant GLUTX expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with GLUTX protein, nucleic acid expression or activity in, *e.g* aberrant hexose transport disorders, including but not limited to ischemia, hyperglycemia, hypoglycemia, diabetes, etc. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant GLUTX expression or activity in which a test sample is obtained from a subject and GLUTX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein the presence of GLUTX protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant GLUTX expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant GLUTX expression or activity. For example, such methods can be

used to determine whether a subject can be effectively treated with an agent for a disorder, such as a aberrant hexose transport disorders, including but not limited to ischemia, hyperglycemia, hypoglycemia, diabetes, etc. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant GLUTX expression or activity in which a test sample is obtained and GLUTX protein or nucleic acid is detected (*e.g.*, wherein the presence of GLUTX protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant GLUTX expression or activity.)

The methods of the invention can also be used to detect genetic lesions in a GLUTX gene, thereby determining if a subject with the lesioned gene is at risk for, or suffers from, a aberrant hexose transport disorders, including but not limited to ischemia, hyperglycemia, hypoglycemia, diabetes, etc. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding a GLUTX-protein, or the mis-expression of the GLUTX gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of (1) a deletion of one or more nucleotides from a GLUTX gene; (2) an addition of one or more nucleotides to a GLUTX gene; (3) a substitution of one or more nucleotides of a GLUTX gene, (4) a chromosomal rearrangement of a GLUTX gene; (5) an alteration in the level of a messenger RNA transcript of a GLUTX gene, (6) aberrant modification of a GLUTX gene, such as of the methylation pattern of the genomic DNA, (7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a GLUTX gene, (8) a non-wild type level of a GLUTX-protein, (9) allelic loss of a GLUTX gene, and (10) inappropriate post-translational modification of a GLUTX-protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in a GLUTX gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Pat. Nos. 4,683,195 and 4,683,202), such as anchor PCR or "rapid amplification of cDNA ends" (RACE PCR), or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *PNAS* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the GLUTX-gene (see Abravaya *et al.* (1995) *Nucl Acids Res*

23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to a GLUTX gene under conditions such that hybridization and amplification of the GLUTX gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli *et al.*, 1990, *Proc Natl Acad Sci USA* 87:1874-1878), transcriptional amplification system (Kwoh, *et al.*, 1989, *Proc Natl Acad Sci USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al*, 1988, *BioTechnology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a GLUTX gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Pat. No. 5,493,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in GLUTX can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin *et al.* (1996) *Human Mutation* 7: 244-255; Kozal *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in GLUTX can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin *et al.* above. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed

of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the GLUTX gene and detect mutations by comparing the sequence of the sample GLUTX with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert (1977) *PNAS* 74:560 or Sanger (1977) *PNAS* 74:5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (Naeve *et al.*, (1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publ. No. WO 94/16101; Cohen *et al.* (1996) *Adv Chromatogr* 36:127-162; and Griffin *et al.* (1993) *Appl Biochem Biotechnol* 38:147-159).

Other methods for detecting mutations in the GLUTX gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type GLUTX sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al* (1988) *Proc Natl Acad Sci USA* 85:4397; Saleeba *et al* (1992) *Methods Enzymol* 217:286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in GLUTX cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a GLUTX sequence, *e.g.*, a wild-type GLUTX

sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Pat. No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in GLUTX genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl Acad Sci USA*: 86:2766, see also Cotton (1993) *Mutat Res* 285:125-144; Hayashi (1992) *Genet Anal Tech Appl* 9:73-79). Single-stranded DNA fragments of sample and control GLUTX nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA, rather than DNA, in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility. See, *e.g.*, Keen *et al.* (1991) *Trends Genet* 7:5.

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE). See, *e.g.*, Myers *et al* (1985) *Nature* 313:495. When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA. See, *e.g.*, Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753.

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found. See, *e.g.*, Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc Natl Acad. Sci USA* 86:6230. Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection. See, *e.g.*, Gasparini *et al* (1992) *Mol Cell Probes* 6:1. It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification. See, *e.g.*, Barany (1991) *Proc Natl Acad Sci USA* 88:189. In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a GLUTX gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which GLUTX is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on GLUTX activity (*e.g.*, GLUTX gene expression), as identified by a screening assay described herein can be administered to individuals to treat aberrant hexose transport disorders, including but not limited to ischemia, hyperglycemia, hypoglycemia, diabetes, etc., associated with aberrant GLUTX activity. In conjunction with such treatment, the pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (*e.g.*, drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype.

Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of GLUTX protein, expression of GLUTX nucleic acid, or mutation content of GLUTX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*, Eichelbaum, 1996, *Clin Exp Pharmacol Physiol*, 23:983-985 and Linder, 1997, *Clin Chem*, 43:254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of GLUTX protein, expression of GLUTX nucleic acid, or mutation content of GLUTX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition,

pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a GLUTX modulator, such as a modulator identified by one of the exemplary screening assays described herein.

Monitoring Clinical Efficacy

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of GLUTX (*e.g.*, the aberrant hexose transport disorders, including but not limited to ischemia, hyperglycemia, hypoglycemia, diabetes, etc.) can be applied in basic drug screening and in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase GLUTX gene expression, protein levels, or upregulate GLUTX activity, can be monitored in clinical trials of subjects exhibiting decreased GLUTX gene expression, protein levels, or downregulated GLUTX activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease GLUTX gene expression, protein levels, or downregulate GLUTX activity, can be monitored in clinical trials of subjects exhibiting increased GLUTX gene expression, protein levels, or upregulated GLUTX activity. In such clinical trials, the expression or activity of GLUTX and, preferably, other genes that have been implicated in, for example, a proliferative or neurological disorder, can be used as a "read out" or marker of the responsiveness of a particular cell.

For example, genes, including GLUTX, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates GLUTX activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on aberrant hexose transport disorders, including but not limited to ischemia, hyperglycemia, hypoglycemia, diabetes, etc., for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of GLUTX and other genes implicated in the disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of GLUTX or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In one embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide, nucleic acid, peptidomimetic, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (*i*) obtaining a pre-administration sample from a subject prior to administration of the agent; (*ii*) detecting the level of expression of a GLUTX protein, mRNA, or genomic DNA in the preadministration sample; (*iii*) obtaining one or more post-administration samples from the subject; (*iv*) detecting the level of expression or activity of the GLUTX protein, mRNA, or genomic DNA in the post-administration samples; (*v*) comparing the level of expression or activity of the GLUTX protein, mRNA, or genomic DNA in the pre-administration sample with the GLUTX protein, mRNA, or genomic DNA in the post administration sample or samples; and (*vi*) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of GLUTX to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of GLUTX to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant GLUTX expression or activity.

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, (*i*) a GLUTX polypeptide, or analogs, derivatives, fragments or homologs thereof; (*ii*) antibodies to a GLUTX peptide; (*iii*) nucleic acids encoding a GLUTX peptide; (*iv*) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences to a GLUTX peptide) that are utilized to "knockout" endogenous function of a GLUTX peptide by homologous recombination (see, *e.g.*, Capecchi, 1989, *Science* 244: 1288-1292); or (*v*) modulators (*i.e.*, inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between a GLUTX peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, a GLUTX peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of a GLUTX peptide). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, etc.).

In one aspect, the invention provides a method for preventing, in a subject, a disease or condition associated with an aberrant GLUTX expression or activity, by administering to the subject an agent that modulates GLUTX expression or at least one GLUTX activity. Subjects at risk for a disease that is caused or contributed to by aberrant GLUTX expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the GLUTX aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of GLUTX aberrancy, for example, a GLUTX agonist or GLUTX antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

Another aspect of the invention pertains to methods of modulating GLUTX expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of GLUTX protein activity associated with the cell. An agent that modulates GLUTX protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of a GLUTX protein, a peptide, a GLUTX peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more GLUTX protein activity. Examples of such stimulatory agents include active GLUTX protein and a nucleic acid molecule encoding GLUTX that has been introduced into the cell. In another embodiment, the agent inhibits one

or more GLUTX protein activity. Examples of such inhibitory agents include antisense GLUTX nucleic acid molecules and anti-GLUTX antibodies. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a GLUTX protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) GLUTX expression or activity. In another embodiment, the method involves administering a GLUTX protein or nucleic acid molecule as therapy to compensate for reduced or aberrant GLUTX expression or activity.

The invention will be further illustrated in the following examples, which do not limit the scope of the claims.

EXAMPLES

Example 1. Cloning and Sequence Analysis of GLUTX1

Amino acid sequences of human GLUTs 1-5 (Swiss-Prot accession numbers P11166, P11168, P11169, P14672 and P22732) were used to screen the Expressed Sequence Tag (EST) data base at NCBI using TBLASTN. Novel ESTs were analyzed using the Genetics Computer Group program suite. ESTs showing significant homology to the GLUTs were identified.

Human EST clones 50147 and 46121 (accession numbers H18721 and H09414, respectively) encoding the carboxyl terminus and 3'-untranslated region of GLUTX1 were used as probes on rat multiple tissue Northern blots and identified a 2-kilobase transcript that was strongly expressed in testis.

Primers specific for EST H34451 were used to amplify GLUTX1 cDNA by 5'- and 3'-RACE from rat testis poly(A)$^+$ RNA using Roche Molecular Biochemicals 5' and 3'-RACE reagents. PCR products were cloned using the TOPO cloning system (Invitrogen). For amplification of the full-length GLUTX1 sequence, primers were designed from the 5'- and 3'-RACE products and PCR amplification performed using the Expand long template PCR system (Roche Molecular Biochemicals). Three independent reverse transcription-PCR reactions were performed from rat testis poly(A)$^+$ RNA, and the resulting products were cloned and sequenced in both directions. Mouse GLUTX1 cDNA was determined by sequencing 5'- and 3'-RACE products and mouse EST clones. Human GLUTX1 cDNA sequence was determined from sequencing human EST and BAC clones (Research Genetics).

A 2087-base pair cDNA encoding a protein of 478 amino acids was cloned from rat testis mRNA by RACE as described in the EXAMPLES. The amino acid sequence of GLUTX1 compared with eight members of the sugar transport family is presented in FIG. 1. The full length sequence of the human GLUTX1 cDNA was cloned separately. The 5' end was obtained from a BAC clone, thus the sequence upstream of the ATG start codon is part of the gene promoter (see Table 2).

GLUTX1(LL-AA) was constructed from GLUTX1 by mutagenesis using a modified 5' primer. The resulting cDNA was cloned into pcDNA3 (Invitrogen) and pSD5 vectors for expression analyses. See, *e.g.*, Geering *et al.* 1996 *J. Cell Biol.* 133: 1193-1204. Sequence compilations and comparisons were made using the best fit, gap, and pileup programs (Genetics Computer Group program suite) and the ClustalX program. See, *e.g.*, Thompson *et al.* 1997 *Nucleic Acids Res.* 25: 4876-4882.

Alignment of GLUTX1 was made using the pileup (Genetics Computer Group) and ClustalX programs to other members of the GLUT family of proteins, as shown in FIG. 1. See, Ibberson *et al.*, 2000 *J. Biol. Chem.* 275: 4607-4612. Black shading indicates identical residues, and gray shading indicates amino acids belonging to the same conservation group. The approximate positions of the transmembrane domains are marked. The position of a dileucine motif in GLUTX1 sequence is indicated by a horizontal bar, and the position of a putative glycosylation site is indicated by an *asterisk*. Four regions of conservation between GLUTX1 and integral membrane protein are marked with boxes. FIG. 2 shows the alignment of the respective human, rat and mouse sequences for GLUTX1 (Panel A), GLUTX2 (Panel B) and GLUTX3 (Panel C). Additional alignments and consensus/majority sequences are provided in the various Tables below.

Human, rat and mouse GLUTX1 sequences are shown in Tables 3A, 3B and 3C, respectively. An alignment of human (hX1), rat (rX1) and mouse (mX1) GLUTX1 polypeptide sequences is shown in Table 3D. The twelve putative TM domains are approximately delineated by the solid line above the aligned sequence In Table 3D, plus all other sequence alignments, fully conserved single residues are indicated by (*), fully conserved "strong" residues are indicated by (:), and fully conserved "weak" residues are indicated by (.). The "strong" group of conserved amino acid residues may be any one of the following groups: STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW. The "weak" group of conserved residues may be any one of the following: CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, VLIM, HFY. The putative

glycosylated N residue is indicated by (+). The dileucine repeat is indicated by (o). The "Majority" GLUTX1 polypeptide sequence of Table 3D is disclosed as SEQ ID NO:15.

TABLE 3A

Human GLUTX1 Nucleotide (SEQ ID NO:1) and Amino Acid (SEQ ID NO:2) Sequences

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CCCGTCTATG GGCTCGCACT ACACTCCAAT TGGGTGGTTG TTCTCCCAGA CGGAAACTCC   60
GCCCCACCTG TACCTTGGAC CGCTCCGCCC TAGGGCGGTT CCAGCGCGCT TCGCTAATCG  120
GTTCTATGCT CCCATTGGTC TGCTTTAATG TAAGGGCGGG TCCTAGCTGA TTCGCTAGCC  180
CCGCCTTGCG TTCCTATTGG CTGCTCCTGC TCTGCGGTTC CGATCCCGCC CAGCACCGTC  240
CCCCACCCCC CGCCGCCCAT TGGCTGCGAG AGGCCGGTGC GGGCCGCACT CGCAGGGCCC  300

GTGGCGGTTC AGGCGCCAGA GCTGGCCGAT CGGCGTTGGC CGCCGACATG ACGCCCGAGG  360
                                                    M   T   P   E   D

ACCCAGAGGA AACCCAGCCG CTTCTGGGGC CTCCTGGCGG CAGCGCGCCC CGCGGCCGCC  420
  P   E   E   T   Q   P   L   L   G   P   P   G   G   S   A   P   R   G   R   R

GCGTCTTCCT CGCCGCCTTC GCCGCTGCCC TGGGCCCACT CAGCTTCGGC TTCGCGCTCG  480
  V   F   L   A   A   F   A   A   A   L   G   P   L   S   F   G   F   A   L   G

GCTACAGCTC CCCGGCCATC CCTAGCCTGC AGCGCGCCGC GCCCCCGGCC CCGCGCCTGG  540
  Y   S   S   P   A   I   P   S   L   Q   R   A   A   P   P   A   P   R   L   D

ACGACGCCGC CGCCTCCTGG TTCGGGGCTG TCGTGACCCT GGGTGCCGCG GCGGGGGGAG  600
  D   A   A   A   S   W   F   G   A   V   V   T   L   G   A   A   A   G   G   V

TGCTGGGCGG CTGGCTGGTG GACCGCGCCG GGCGCAAGCT GAGCCTCTTG CTGTGCTCCG  660
  L   G   G   W   L   V   D   R   A   G   R   K   L   S   L   L   L   C   S   V

TGCCCTTCGT GGCCGGCTTT GCCGTCATCA CCGCGGCCCA GGACGTGTGG ATGCTGCTGG  720
  P   F   V   A   G   F   A   V   I   T   A   A   Q   D   V   W   M   L   L   G

GGGGCCGCCT CCTCACCGGC CTGGCCTGCG GTGTTGCCTC CCTAGTGGCC CCGGTCTACA  780
  G   R   L   L   T   G   L   A   C   G   V   A   S   L   V   A   P   V   Y   I

TCTCCGAAAT CGCCTACCCA GCAGTCCGGG GGTTGCTCGG CTCCTGTGTG CAGCTAATGG  840
  S   E   I   A   Y   P   A   V   R   G   L   L   G   S   C   V   Q   L   M   V

TCGTCGTCGG CATCCTCCTG GCCTACCTGG CAGGCTGGGT GCTGGAGTGG CGCTGGCTGG  900
  V   V   G   I   L   L   A   Y   L   A   G   W   V   L   E   W   R   W   L   A

CTGTGCTGGG CTGCGTGCCC CCCTCCCTCA TGCTGCTTCT CATGTGCTTC ATGCCCGAGA  960
  V   L   G   C   V   P   P   S   L   M   L   L   L   M   C   F   M   P   E   T

CCCCGCGCTT CCTGCTGACT CAGCACAGGC GCCAGGAGGC CATGGCCGCC CTGCGGTTCC 1020
  P   R   F   L   L   T   Q   H   R   R   Q   E   A   M   A   A   L   R   F   L

TGTGGGGCTC CGAGCAGGGC TGGGAAGACC CCCCCATCGG GGCTGAGCAG AGCTTTCACC 1080
  W   G   S   E   Q   G   W   E   D   P   P   I   G   A   E   Q   S   F   H   L

TGGCCCTGCT GCGGCAGCCC GGCATCTACA AGCCCTTCAT CATCGGCGTC TCCCTGATGG 1140
  A   L   L   R   Q   P   G   I   Y   K   P   F   I   I   G   V   S   L   M   A

CCTTCCAGCA GCTGTCGGGG GTCAACGCCG TCATGTTCTA TGCAGAGACC ATCTTTGAAG 1200
  F   Q   Q   L   S   G   V   N   A   V   M   F   Y   A   E   T   I   F   E   E
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AGGCCAAGTT CAAGGACAGC AGCCTGGCCT CGGTCGTCGT GGGTGTCATC CAGGTGCTGT  1260
  A  K  F   K  D  S   S  L  A  S   V  V  V   G  V  I   Q  V  L  F

TCACAGCTGT GGCGGCTCTC ATCATGGACA GAGCAGGGCG GAGGCTGCTC CTGGTCTTGT  1320
  T  A  V   A  A  L   I  M  D  R   A  G  R   R  L  L   L  V  L  S

CAGGTGTGGT CATGGTGTTC AGCACGAGTG CCTTCGGCGC CTACTTCAAG CTGACCCAGG  1380
  G  V  V   M  V  F   S  T  S  A   F  G  A   Y  F  K   L  T  Q  G

GTGGCCCTGG CAACTCCTCG CACGTGGCCA TCTCGGCGCC TGTCTCTGCA CAGCCTGTTG  1440
  G  P  G   N  S  S   H  V  A  I   S  A  P   V  S  A   Q  P  V  D

ATGCCAGCGT GGGGCTGGCC TGGCTGGCCG TGGGCAACAT GTGCCTCTTC ATCGCCGGCT  1500
  A  S  V   G  L  A   W  L  A  V   G  N  M   C  L  F   I  A  G  F

TTGCGGTGGG CTGGGGGCCC ATCCCCTGGC TCCTCATGTC AGAGATCTTC CCTCTGCATG  1560
  A  V  G   W  G  P   I  P  W  L   L  M  S   E  I  F   P  L  H  V

TCAAGGGCGT GGCGACAGGC ATCTGCGTCC TCACCAACTG GCTCATGGCC TTTCTCGTGA  1620
  K  G  V   A  T  G   I  C  V  L   T  N  W   L  M  A   F  L  V  T

CCAAGGAGTT CAGCAGCCTC ATGGAGGTCC TCAGGCCCTA TGGAGCCTTC TGGCTTGCCT  1680
  K  E  F   S  S  L   M  E  V  L   R  P  Y   G  A  F   W  L  A  S

CCGCTTTCTG CATCTTCAGT GTCCTTTTCA CTTTGTTCTG TGTCCCTGAA ATTAAAGGAA  1740
  A  F  C   I  F  S   V  L  F  T   L  F  C   V  P  E   I  K  G  K

AGACTCTGGA ACAAATCACA GCCCATTTTG AGGGGCGATG ACAGCCACTC ATTAGGGGAT  1800
  T  L  E   Q  I  T   A  H  F  E   G  R

GGAGCAAGCC TGTGACTCCA AGTTGGGCCC AAGCCCAGAG CCCCTGCCTG CCCCAGGGGA  1860
GCCAGAATCC AGCCCCTTGG AGCCTTGGTC TGCAGGGTCC CTCCTTCCTG TCATGCTCCC  1920
TCCAGCCCAT GACCCGGGGC TAGGAGGCTC ACTGCCTCCT GTTCCAGCTC CTGCTGCTGC  1980
TCTGAGGACT CAGGAACACC TTCGAGCTTT GCAGACCTGC GGTCAGCCCT CCATGCGCAA  2040
GACTAAAGCA GCGGAAGAGG AGGTGGGCCT CTAGGATCTT TGTCTTCTGG CTGGAGGTGC  2100
TTTTGGAGGT TGGGTGCTGG GCATTCGGTC GCTCCTCTCA CGCGGCTGCC TTATCGGGAA  2160
GGAAATTTGT TTGCCAAATA AAGACTGACA CAGAAAATCA AAAAAAAAAA AAAAAAA     2217
```

TABLE 3B

Rat GLUTX1 Nucleotide (SEQ ID NO:3) and Amino Acid (SEQ ID NO:4) Sequences

```
cgacagttcg cgggtcagac cctgttgaca tgtcgcccga ggacccccag gaaacacagc  60
                              M   S  P  E   D  P  Q   E  T  Q  P

cgctgttgcg gtcgccgggc gccagggctc ccggcggccg ccgggtcttc cttgccacct  120
  L  L  R   S  P  G   A  R  A  P   G  G  R   R  V  F   L  A  T  F

tcgccgccgc cctgggcccg ctcagcttcg gcttcgcgct tggctacagc tcccccgcca  180
  A  A  A   L  G  P   L  S  F  G   F  A  L   G  Y  S   S  P  A  I

tccccagcct gcggcgcacc gcacccccctg ccctacgcct cggagacact gcggcctcct  240
  P  S  L   R  R  T   A  P  P  A   L  R  L   G  D  T   A  A  S  W

ggttcggggc cgtcgtgacc ctgggcgctg cggcaggggg cgtgctgggc ggctggctcc  300
  F  G  A   V  V  T   L  G  A  A   A  G  G   V  L  G   G  W  L  L

tggaccgtgc agggcgcaag ctgagcctcc tgctctgcac cgtgcccttc gtgaccggtt  360
```

```
  D  R  A  G  R  K  L  S  L  L  L  C  T  V  P  F  V  T  G  F

ttgctgtcat caccgcggcc cgggatgtgt ggatgctgct cggaggccgc ctcctcaccg  420
  A  V  I  T  A  A  R  D  V  W  M  L  L  G  G  R  L  L  T  G

gcctagcctg cggagtcgcc tcattagtgg caccggtcta tatctcggaa atcgcctacc  480
  L  A  C  G  V  A  S  L  V  A  P  V  Y  I  S  E  I  A  Y  P

cagccgttcg aggactgctc ggctcctgtg tgcagctgat ggttgtcact ggcatcctct  540
  A  V  R  G  L  L  G  S  C  V  Q  L  M  V  V  T  G  I  L  L

tggcctacgt ggcaggctgg gtcctagaat ggcgctggtt ggctgtgctg ggctgtgtgc  600
  A  Y  V  A  G  W  V  L  E  W  R  W  L  A  V  L  G  C  V  P

cccccaccct catgctgctg ctcatgtgct acatgcccga gaccccacgc ttcctcctga  660
  P  T  L  M  L  L  L  M  C  Y  M  P  E  T  P  R  F  L  L  T

cgcaacacca gtaccaggag gccatggctg ccctgcgctt cctgtggggc tctgaggagg  720
  Q  H  Q  Y  Q  E  A  M  A  A  L  R  F  L  W  G  S  E  E  G

gctgggaaga gccccctgtt ggggctgagc accagggctt ccagctggct atgctgaggc  780
  W  E  E  P  P  V  G  A  E  H  Q  G  F  Q  L  A  M  L  R  R

gccctggtgt ccacaagccc ctcatcatcg gcatttgcct catggtcttc cagcagctgt  840
  P  G  V  H  K  P  L  I  I  G  I  C  L  M  V  F  Q  Q  L  S

caggggtcaa cgccatcatg ttctatgcca acaccatctt tgaggaggcc aagttcaagg  900
  G  V  N  A  I  M  F  Y  A  N  T  I  F  E  E  A  K  F  K  D

acagcagcct ggcctcggtc actgtgggca tcatccaggt tctgttcact gctgtggcgg  960
  S  S  L  A  S  V  T  V  G  I  I  Q  V  L  F  T  A  V  A  A

ccctcatcat ggacagagca gggcgaaaac tgcttctggc cttgtcgggt gtgatcatgg 1020
  L  I  M  D  R  A  G  R  K  L  L  L  A  L  S  G  V  I  M  V

ttttcagcat gagcgccttt ggtacctact tcaaactgac ccagagtggc cccagcaact 1080
  F  S  M  S  A  F  G  T  Y  F  K  L  T  Q  S  G  P  S  N  S

cctcccatgt aggcctcctg gtgcccatct ccgcagagcc tgctgatgtt cacctggggc 1140
  S  H  V  G  L  L  V  P  I  S  A  E  P  A  D  V  H  L  G  L

tggcctggct ggctgtaggc agcatgtgcc tcttcatcgc tggttttgca gtaggctggg 1200
  A  W  L  A  V  G  S  M  C  L  F  I  A  G  F  A  V  G  W  G

gacccatccc ctggctcctc atgtcagaga tcttccctct gcacatcaag ggtgtggcta 1260
  P  I  P  W  L  L  M  S  E  I  F  P  L  H  I  K  G  V  A  T

ccggcgtctg tgtcctcacc aactggttca tggcctttct ggtgaccaaa gagtttaaca 1320
  G  V  C  V  L  T  N  W  F  M  A  F  L  V  T  K  E  F  N  S

gcatcatgga gatcctcaga ccctacggcg ccttctggct caccgctgcc ttctgtatcc 1380
  I  M  E  I  L  R  P  Y  G  A  F  W  L  T  A  A  F  C  I  L

tcagcgtcct tttcacgctc acctttgtcc ctgagactaa aggcaggact ctggaacaaa 1440
  S  V  L  F  T  L  T  F  V  P  E  T  K  G  R  T  L  E  Q  I

tcacagccca tttcgaggga cggtgacgga ccctttctgt gactggcagc cctgagctga 1500
  T  A  H  F  E  G  R
```

```
gctggcttcg ggtttcaaaa ggagtggagt ggcctcagtg accacagttt gagcccaggg  1560
gccccctgac tcctcagatc tccgggccag ctttgtccag atctcaaccc agatccacac  1620
catgagcttc accagattct gaggctctgc agcctgctgc acacacagca catctgcgga  1680
gccatgcagt ctcgcctaac ccacgggtct ccttcctaag actaaagcaa ctgacgagac  1740
tgcgggctcc tggctctagt gctctggctg ggcatctttg gggtgcttgg tcctaagcaa  1800
ctgcccatac ctcacttgac tgggggatga gaaagggact tagccacata agatttgggc  1860
tcagaaacaa ggtcaggtga gtccaggaag aaaagagaat gttcttgtct tgtcaaccaa  1920
gtccttctca gagtgcccag agacctccgg attcaccttg gggttagcca gcttacccat  1980
cacttacagg ttctctccaa ctcttccctg gtctcagtgt cctggatcat tagtcaccag  2040
gtcttgttga gtttcagaaa ataaaaggcc tctttccgtt caacttt                2087
```

TABLE 3C

Mouse GLUTX1 Nucleotide (SEQ ID NO:5) and Amino Acid (SEQ ID NO:6) Sequences

```
GCGGGTCAGG TCCTGCTGAC ATGTCTCCCG AGGACCCCCA GGAGACGCAG CCGCTATTGC  60
                      M  S  P  E  D  P  Q  E  T  Q  P  L  L  R

GGCCACCGGA AGCCAGGACT CCCCGCGGCC GCCGGGTCTT CCTCGCTTCC TTCGCCGCCG  120
  P  P  E  A  R  T  P  R  G  R  R  V  F  L  A  S  F  A  A  A

CTCTGGGACC CCTCAACTTC GGCTTCGCGC TCGGCTACAG CTCCCCCGCC ATCCCCAGCC  180
  L  G  P  L  N  F  G  F  A  L  G  Y  S  S  P  A  I  P  S  L

TGCGGCGCAC CGCACCCCCG GCCCTGCGCC TCGGAGACAA TGCGGCCTCC TGGTTCGGGG  240
  R  R  T  A  P  P  A  L  R  L  G  D  N  A  A  S  W  F  G  A

CCGTCGTGAC CCTGGGCGCT GCTGCAGGGG GCATACTGGG CGGCTGGCTC CTGGACCGTT  300
  V  V  T  L  G  A  A  A  G  G  I  L  G  G  W  L  L  D  R  S

CAGGGCGCAA GCTGAGCCTC TTGCTCTGCA CCGTGCCCTT CGTGACTGGC TTTGCTGTCA  360
  G  R  K  L  S  L  L  L  C  T  V  P  F  V  T  G  F  A  V  I

TCACCGCGGC CCGGGATGTG TGGATGCTGC TCGGAGGCCG CCTCCTCACC GGCCTTGCCT  420
  T  A  A  R  D  V  W  M  L  L  G  G  R  L  L  T  G  L  A  C

GCGGAGTCGC CTCACTAGTG GCACCGGTTT ACATCTCGGA AATCGCCTAC CCAGCTGTCC  480
  G  V  A  S  L  V  A  P  V  Y  I  S  E  I  A  Y  P  A  V  R

GAGGACTGCT CGGCTCCTGT GTGCAGCTGA TGGTTGTCAC TGGCATCCTC CTGGCCTATG  540
  G  L  L  G  S  C  V  Q  L  M  V  V  T  G  I  L  L  A  Y  V

TGGCAGGCTG GGTCCTAGAG TGGCGCTGGC TGGCCGTGCT GGGCTGTGTG CCCCCCACCC  600
  A  G  W  V  L  E  W  R  W  L  A  V  L  G  C  V  P  P  T  L

TCATGCTGCT GCTCATGTGC TACATGCCCG AGACCCCACG TTTTCTCCTC ACTCAACACC  660
  M  L  L  L  M  C  Y  M  P  E  T  P  R  F  L  L  T  Q  H  Q

AGTACCAGGA GGCCATGGCT GCCTTGCGCT TCCTGTGGGG CTCTGAGGAG GGCTGGGAAG  720
  Y  Q  E  A  M  A  A  L  R  F  L  W  G  S  E  E  G  W  E  E

AGCCCCCTGT TGGGGCTGAG CACCAGGGCT TCCAGCTGGC CCTGCTGAGG CGCCCTGGCA  780
  P  P  V  G  A  E  H  Q  G  F  Q  L  A  L  L  R  R  P  G  I

TCTACAAGCC CCTCATCATC GGCATTTCCC TCATGGTCTT CCAGCAGCTG TCAGGGGTCA  840
  Y  K  P  L  I  I  G  I  S  L  M  V  F  Q  Q  L  S  G  V  N

ATGCTATCAT GTTCTATGCC AACAGCATCT TCGAGGAGGC CAAGTTCAAG GACAGCAGCC  900
```

```
  A  I  M  F  Y  A  N  S  I  F  E  E  A  K  F  K  D  S  S  L

TGGCCTCGGT CACTGTGGGC ATAATCCAGG TCCTGTTCAC TGCTGTGGCG GCCCTCATCA  960
  A  S  V  T  V  G  I  I  Q  V  L  F  T  A  V  A  A  L  I  M

TGGACAGAGC AGGGCGAAGG CTGCTCCTGG CCTTGTCGGG TGTGATCATG GTGTTCAGTA  1020
  D  R  A  G  R  R  L  L  L  A  L  S  G  V  I  M  V  F  S  M

TGAGTGCCTT TGGTACCTAC TTCAAACTGA CCCAGAGCCT CCCCAGCAAC TCCTCCCACG  1080
  S  A  F  G  T  Y  F  K  L  T  Q  S  L  P  S  N  S  S  H  V

TAGGCCTGGT GCCCATCGCG GCGGAGCCTG TGGATGTCCA AGTGGGACTG GCCTGGCTGG  1140
  G  L  V  P  I  A  A  E  P  V  D  V  Q  V  G  L  A  W  L  A

CTGTAGGCAG CATGTGCCTC TTCATTGCTG GCTTTGCGGT GGGCTGGGGA CCCATCCCCT  1200
  V  G  S  M  C  L  F  I  A  G  F  A  V  G  W  G  P  I  P  W

GGCTCCTCAT GTCAGAGATC TTCCCTCTGC ATGTCAAGGG TGTGGCTACC GGCATCTGTG  1260
  L  L  M  S  E  I  F  P  L  H  V  K  G  V  A  T  G  I  C  V

TCCTCACCAA CTGGTTCATG GCCTTTCTAG TGACCAAAGA GTTCAACAGC GTCATGGAGA  1320
  L  T  N  W  F  M  A  F  L  V  T  K  E  F  N  S  V  M  E  M

TGCTCAGACC CTACGGTGCC TTCTGGCTCA CCGCTGCCTT CTGCGCTCTC AGTGTCCTAT  1380
  L  R  P  Y  G  A  F  W  L  T  A  A  F  C  A  L  S  V  L  F

TCACACTGAC CGTTGTCCCT GAGACTAAAG GCAGGACTCT GGAACAAGTC ACAGCCCATT  1440
  T  L  T  V  V  P  E  T  K  G  R  T  L  E  Q  V  T  A  H  F

TCGAGGGACG ATGACAGACC CTTTCTGTGC CTGGGAGCCC CGAGCTGAGC TGCCTTTGGG  1500
  E  G  R

TTTCAGAAGG AGTGGAGTGG CCTGTAACTA AGCCACACCT CAGTTTGAGC CTGGAGGCCC  1560
CTGACTCCTC ACCTCAGGGC CCTCTTTGCC CAGATCTCAA CCCAGATTCC CACCGTGAGC  1620
CCACCAGACT CTGAGTGCGG TCTCTGCAGC CTGCTTCACA CACAGGACAT CTGAGGAGCT  1680
GTGCATCCTC ACCTGACCCA GGTGTCTCCT TCTTAAGACT GAGGCAGCTG AAGGGACCTG  1740
AGGGCTCCTG GCTCCCGTTT CCTGGCTGGG GTGCCCGGTC CTAAGCAGCC GCCTGTACCT  1800
CACTTGACTG GGAGATCAGA AAGGGACTTA GCCATATGGG CTCAGAAACA AGGTCAGGTG  1860
AGCCCAGGTA GAAGAGAGAA TGTTCTTGCC AACCAAGCCC TCCTCAGAGC TGCGCAGAGA  1920
CTCTCCGGGG TCACCCTGGG GCCAGCCAGC TTACCCATCA CTTACAGGTT CTTTCTGGCT  1980
CTTTCCTGGG CTCCGTGTCC TGGGTCATTA GCCACCATAT CTTGTTGAGT TTCAGGAAAT  2040
AAAAAGCCTC TTACTGTTCA AAAAAAAACA AA                                 2072
```

TABLE 3D

Sequence alignment of rat, human and mouse GLUTX1 polypeptides and Majority GLUTX1 Sequence (SEQ ID NO:15)

```
                        oo                  _________TM1_________
mX1          MSPEDPQETQPLLRPPEARTPRGRRVFLASFAAALGPLNFGFALGYSSPAIPSLRRTAPP
rX1          MSPEDPQETQPLLRSPGARAPGGRRVFLATFAAALGPLSFGFALGYSSPAIPSLRRTAPP
hX1          MTPEDPEETQPLLGPPGGSAPRGRRVFLAAFAAALGPLSFGFALGYSSPAIPSLQRAAPP
             *:****:****** .* . :* *******:********.***************:*:***
Majority     MsPEDPqETQPLLrpPgaraPrGRRVFLAaFAAALGPLsFGFALBYSSPAIPSLrRtAPP

                        _______TM2_____              _________TM3______
mX1          ALRLGDNAASWFGAVVTLGAAAGGILGGWLLDRSGRKLSLLLCTVPFVTGFAVITAARDV
rX1          ALRLGDTAASWFGAVVTLGAAAGGVLGGWLLDRAGRKLSLLLCTVPFVTGFAVITAARDV
hX1          APRLDDAAASWFGAVVTLGAAAGGVLGGWLVDRAGRKLSLLLCSVPFVAGFAVITAAQDV
```

```
                * **.* ******************:*****:**:*********:****:********:**
Majority        AlRLgDaAASWFGAVVTLGAAAGGvLGGWLlDRaGRKLSLLLCtVPFVtGFAVITAArDV

                           ___________TM4_________          ____________TM5________
mX1             WMLLGGRLLTGLACGVASLVAPVYISEIAYPAVRGLLGSCVQLMVVTGILLAYVAGWVLE
rX1             WMLLGGRLLTGLACGVASLVAPVYISEIAYPAVRGLLGSCVQLMVVTGILLAYVAGWVLE
hX1             WMLLGGRLLTGLACGVASLVAPVYISEIAYPAVRGLLGSCVQLMVVVGILLAYLAGWVLE
                ********************************************.******:******
Majority        WMLLGGRLLTGLACGVASLVAPVYISEIAYPAVRGLLGSCVQLMVVtGILLAYvAGWVLE

                _________TM6___________
mX1             WRWLAVLGCVPPTLMLLLMCYMPETPRFLLTQHQYQEAMAALRFLWGSEEGWEEPPVGAE
rX1             WRWLAVLGCVPPTLMLLLMCYMPETPRFLLTQHQYQEAMAALRFLWGSEEGWEEPPVGAE
hX1             WRWLAVLGCVPPSLMLLLMCFMPETPRFLLTQHRRQEAMAALRFLWGSEQGWEDPPIGAE
                ************:*******:************: ***************:***:**:***
Majority        WRWLAVLGCVPPtLMLLLMCyMPETPRFLLTQHqyQEAMAALRFLWGSEeGWEePPvGAE

                                    ___________TM7__________              ________
mX1             HQGFQLALLRRPGIYKPLIIGISLMVFQQLSGVNAIMFYANSIFEEAKFKDSSLASVTVG
rX1             HQGFQLAMLRRPGVHKPLIIGICLMVFQQLSGVNAIMFYANTIFEEAKFKDSSLASVTVG
hX1             QS-FHLALLRQPGIYKPFIIGVSLMAFQQLSGVNAVMFYAETIFEEAKFKDSSLASVVVG
                :. *:**:**:**::**:***:.**.*********:****::***************.**
Majority        hqgFqLAlLRrPGiyKPlIIGisLMvFQQLSGVNAiMFYAntIFEEAKFKDSSLASVtVG

                __TM8_________                ___________TM9_________     +
mX1             IIQVLFTAVAALIMDRAGRRLLLALSGVIMVFSMSAFGTYFKLTQSLPSNSSHVG-LVPI
rX1             IIQVLFTAVAALIMDRAGRKLLLALSGVIMVFSMSAFGTYFKLTQSGPSNSSHVGLLVPI
hX1             VIQVLFTAVAALIMDRAGRRLLLVLSGVVMVFSTSAFGAYFKLTQGGPGNSSHVAISAPV
                :*******************:***.****:**** ****:******. *.*****.  .*:
Majority        iIQVLFTAVAALIMDRAGRrLLLaLSGViMVFSmSAFGtYFKLTQsgPsNSSHVgilvPi

                                  _________TM10_________              _______TM11__
mX1             AAEPVDVQVGLAWLAVGSMCLFIAGFAVGWGPIPWLLMSEIFPLHVKGVATGICVLTNWF
rX1             SAEPADVHLGLAWLAVGSMCLFIAGFAVGWGPIPWLLMSEIFPLHIKGVATGVCVLTNWF
hX1             SAQPVDASVGLAWLAVGNMCLFIAGFAVGWGPIPWLLMSEIFPLHVKGVATGICVLTNWL
                :*:*.*. :********.****************************:******:******:
Majority        sAePvDvsvGLAWLAVGsMCLFIAGFAVGWGPIPWLLMSEIFPLHvKGVATGiCVLTNWf

                _________          __________TM12_________
mX1             MAFLVTKEFNSVMEMLRPYGAFWLTAAFCALSVLFTLTVVPETKGRTLEQVTAHFEGR
rX1             MAFLVTKEFNSIMEILRPYGAFWLTAAFCILSVLFTLTFVPETKGRTLEQITAHFEGR
hX1             MAFLVTKEFSSLMEVLRPYGAFWLASAFCIFSVLFTLFCVPEIKGKTLEQITAHFEGR
                *********.*:**:**********::*** :******  *** **:****:********
Majority        MAFLVTKEFnSlMEvLRPYGAFWLtaAFCilSVLFTLtcVPEtKGrTLEQiTAHFEGR
```

Similar methodologies were used to clone human and rat GLUTX2, as shown in Table 4A and 4B. An alignment of human (hX2) and rat (rX2) GLUTX2 is shown in Table 4C. The twelve putative TM domains are approximately delineated by the solid line above the aligned sequence. In Table 4C, fully conserved single residues are indicated by (*), fully conserved "strong" residues are indicated by (:), and fully conserved "weak" residues are indicated by (.). The putative glycosylated N residue is indicated by (+).The dileucine repeat is indicated by (o). The "Majority" GLUTX2 polypeptide sequence is disclosed as SEQ ID NO:16.

TABLE 4A

Human GLUTX2 Polynucleotide (SEQ ID NO:7) and Amino Acid (SEQ ID NO:8) Sequences

Sequence Range: 1 to 2177; Coding Region: 288 to 2174

```
   1  actatagggc gaattgggta ccgggccccc cctcgaggtc gacggtatcg ataagcttga
  61  tatcgaattc cggccgtggc ggcggctgct gccgtggcag ccggagcgga agccgggagg
 121  aagaaagcgg cggcagcggc ggttgctccc gccggctcgg gctgtctagc tcgccgagac
 181  tgccggcccg cggagccgcg tccccccggg cagccccggg cccctgccct atgtcccgca

 241  aggcaagcga gaatgtggag tacacgctgc ggagcctgag cagcctgatg ggcgagcggc
                                                      M   G   E   R   R

 301  gcaggaagca gccggagccg gacgcggcga gcgcggccgg ggagtgcagc ctcctggctg
        R   K   Q   P   E   P   D   A   A   S   A   A   G   E   C   S   L   L   A   A

 361  ccgccgaatc gagcaccagc ctgcagagcg cgggcgcggg cggcggcggc gtcggggacc
        A   E   S   S   T   S   L   Q   S   A   G   A   G   G   G   G   V   G   D   L

 421  tggagcgcgc ggcgcggcgg cagttccagc aggacgagac ccccgccttc gtgtacgtgg
        E   R   A   A   R   R   Q   F   Q   Q   D   E   T   P   A   F   V   Y   V   V

 481  tggccgtctt ctccgcgctg ggcggcttcc tgtttggcta tgacaccggg gtggtgtcag
        A   V   F   S   A   L   G   G   F   L   F   G   Y   D   T   G   V   V   S   G

 541  gggccatgct gctgctcaag cggcagctca gtctggacgc gctgtggcag gagctgctgg
        A   M   L   L   L   K   R   Q   L   S   L   D   A   L   W   Q   E   L   L   V

 601  tgtccagcac ggtgggggcg gctgccgtct cggcgctggc cggaggcgcc ctcaacggcg
        S   S   T   V   G   A   A   A   V   S   A   L   A   G   G   A   L   N   G   V

 661  tcttcggccg ccgcgctgcc atcctcctgg ccagtgccct cttcaccgcc ggctccgcgg
        F   G   R   R   A   A   I   L   L   A   S   A   L   F   T   A   G   S   A   V

 721  tgctggctgc ggccaacaac aaggagacac tgctcgccgg ccgcctggtc gtgggactcg
        L   A   A   A   N   N   K   E   T   L   L   A   G   R   L   V   V   G   L   G

 781  gcatcggcat tgcttctatg acagtgccag tgtacattgc ggaggtctca ccacccaatt
        I   G   I   A   S   M   T   V   P   V   Y   I   A   E   V   S   P   P   N   L

 841  taagaggccg attagtcacc attaataccc tcttcatcac aggagggcag ttctttgcaa
        R   G   R   L   V   T   I   N   T   L   F   I   T   G   G   Q   F   F   A   S

 901  gtgttgttga tggagccttc agttatctcc agaaggatgg atggaggtac atgttgggac
        V   V   D   G   A   F   S   Y   L   Q   K   D   G   W   R   Y   M   L   G   L

 961  ttgcagcagt tccggcggtt atacagtttt ttggctttct ctttttgcct gaaagccctc
        A   A   V   P   A   V   I   Q   F   F   G   F   L   F   L   P   E   S   P   R

1021  gatggcttat tcagaaagga cagactcaga aggcccgtag aattttatct cagatgcgtg
        W   L   I   Q   K   G   Q   T   Q   K   A   R   R   I   L   S   Q   M   R   G

1081  gtaaccagac cattgatgag gaatatgata gcatcaaaaa caacattgaa gaggaggaaa
        N   Q   T   I   D   E   E   Y   D   S   I   K   N   N   I   E   E   E   E   K

1141  aagaggttgg ctcagctgga cctgtgatct gcagaatgct gagttatccc ccaactcgcc
        E   V   G   S   A   G   P   V   I   C   R   M   L   S   Y   P   P   T   R   R

1201  gagctttaat tgtgggttgt ggcctacaaa tgttccagca gctctcaggc attaacacca
        A   L   I   V   G   C   G   L   Q   M   F   Q   Q   L   S   G   I   N   T   I
```

```
1261  tcatgtacta cagtgcaacc attctgcaga tgtctggtgt tgaagatgat agacttgcaa
        M  Y  Y   S  A  T   I  L  Q  M   S  G  V   E  D  D   R  L  A  I

1321  tatggctggc ttcagttaca gccttcacaa atttcatttt cacacttgtg ggagtctggc
        W  L  A   S  V  T   A  F  T  N   F  I  F   T  L  V   G  V  W  L

1381  ttgttgagaa ggtgggccgc agaaagctta cctttggtag tttagcaggt accaccgtag
        V  E  K   V  G  R   R  K  L  T   F  G  S   L  A  G   T  T  V  A

1441  cactcattat tcttgccttg ggatttgtgc tatcagccca agtttcccca cgcatcactt
        L  I  I   L  A  L   G  F  V  L   S  A  Q   V  S  P   R  I  T  F

1501  ttaagccaat agctccgtca ggtcagaacg ccacttgcac aagatacagt tactgtaatg
        K  P  I   A  P  S   G  Q  N  A   T  C  T   R  Y  S   Y  C  N  E

1561  aatgtatgtt ggatccagac tgcggtttct gctacaagat gaacaaatca actgtcattg
        C  M  L   D  P  D   C  G  F  C   Y  K  M   N  K  S   T  V  I  D

1621  actcctcctg tgttccagtt aataaagcat ctacaaatga ggcagcctgg ggcaggtgtg
        S  S  C   V  P  V   N  K  A  S   T  N  E   A  A  W   G  R  C  E

1681  aaaatgaaac caagttcaaa acagaagata tattttgggc ttacaatttc tgccctactc
        N  E  T   K  F  K   T  E  D  I   F  W  A   Y  N  F   C  P  T  P

1741  catactcctg gactgcactt ctgggcctta ttttatatct tgtcttcttt gcacctggaa
        Y  S  W   T  A  L   L  G  L  I   L  Y  L   V  F  F   A  P  G  M

1801  tgggaccaat gccttggact gtgaattctg aaatatatcc cctttgggca agaagtacag
        G  P  M   P  W  T   V  N  S  E   I  Y  P   L  W  A   R  S  T  G

1861  gaaatgcatg ttcatctgga ataaactgga ttttcaatgt cctggtttca ctaacatttt
        N  A  C   S  S  G   I  N  W  I   F  N  V   L  V  S   L  T  F  L

1921  tacacacagc agagtatctt acatactatg gagctttctt cctctatgct ggatttgctg
        H  T  A   E  Y  L   T  Y  Y  G   A  F  F   L  Y  A   G  F  A  A

1981  ctgtgggact ccttttcatc tatggctgtc ttcctgagac caaaggcaaa aaattagagg
        V  G  L   L  F  I   Y  G  C  L   P  E  T   K  G  K   K  L  E  E

2041  aaattgaatc actctttgac aacaggctat gtacatgtgg cacttcagat tctgatgaag
        I  E  S   L  F  D   N  R  L  C   T  C  G   T  S  D   S  D  E  G

2101  ggagatatat tgaatatatc cgcgtgaagg gaagtaacta ccatctctcc gacaacgacg
        R  Y  I   E  Y  I   R  V  K  G   S  N  Y   H  L  S   D  N  D  A

2161  cctcggacgt ggagtga
        S  D  V   E
```

TABLE 4B

Rat GLUTX2 Polynucleotide (SEQ ID NO:9) and Amino Acid (SEQ ID NO:10) Sequences

```
GGCACGAGGC GGCTCAGCAG CTGGCAGCGA GTGCGCGGCG CCCCACGGAA CTTCCCCGCA  60
GCCGCCCGGG CGGCGTCCGT CTGTCCCATG TCCCGCAAGG CGAGCGAGGA CGTGGAGTAC  120

ACGCTGCGCA GCCTGAGCAG CCTGATGGGC GAGAGGCGCC GCCGGCAGCC GGAGCCCGGG  180
                        M  G   E  R  R  R   R  Q  P   E  P  G

GCGCCGGGCG GGGAGCGCAG CCTCCTGGCC GCCGAGTCGG CCGCCAGCCT GCAGGGCGCG  240
A  P  G  G   E  R  S   L  L  A   A  E  S  A   A  S  L   Q  G  A
```

```
GAGCTGGAGC GCGCGGCGCG CAGGCAGTTC CAGCGCGACG AGACCCCCGC CTTCGTGTAC  300
E  L  E  R  A  A  R  R  Q  F  Q  R  D  E  T  P  A  F  V  Y

GCGGCCGCCG CCTTCTCCGC TCTCGGCGGC TTCCTGTTCG GCTACGACAC CGGCGTGGTG  360
A  A  A  A  F  S  A  L  G  G  F  L  F  G  Y  D  T  G  V  V

TCGGGGGCCA TGCTGCTGCT GCGGCGCCAG ATGCGCCTGG GCGCGATGTG GCAGGAGCTG  420
S  G  A  M  L  L  L  R  R  Q  M  R  L  G  A  M  W  Q  E  L

CTGGTGTCGG GCGCGGTGGG CGCGGCCGCC GTCGCGGCGC TGGCCGGAGG GGCCCTGAAC  480
L  V  S  G  A  V  G  A  A  A  V  A  A  L  A  G  G  A  L  N

GGCGCCCTCG GTCGGCGAAG CGCCATCCTG CTGGCCAGCG CCCTGTGCAC CGTGGGCTCC  540
G  A  L  G  R  R  S  A  I  L  L  A  S  A  L  C  T  V  G  S

GCCGTGCTGG CCGCCGCCGC CAACAAGGAG ACGCTGCTGG CCGGCCGCCT GGTCGTGGGG  600
A  V  L  A  A  A  A  N  K  E  T  L  L  A  G  R  L  V  V  G

CTCGGCATCG GCATCGCATC CATGACAGTG CCCGTGTACA TCGCCGAGGT CTCTCCACCC  660
L  G  I  G  I  A  S  M  T  V  P  V  Y  I  A  E  V  S  P  P

AACCTGAGAG GTCGCTTGGT CACCATCAAC ACCCTCTTCA TCACCGGCGG ACAGTTCTTT  720
N  L  R  G  R  L  V  T  I  N  T  L  F  I  T  G  G  Q  F  F

GCGAGCGTTG TTGATGGAGC CTTTAGTTAC CTGCAGAAGG ATGGATGGAG GTACATGTTG  780
A  S  V  V  D  G  A  F  S  Y  L  Q  K  D  G  W  R  Y  M  L

GGACTTGCGG CCATTCCAGC CGTTATACAA TTCCTCGGAT TCCTCTTTTT GCCCGAAAGT  840
G  L  A  A  I  P  A  V  I  Q  F  L  G  F  L  F  L  P  E  S

CCTCGGTGGC TGATACAGAA AGGACAGACT CAGAAGGCCC GCCGAATTTT GTCCCAGATG  900
P  R  W  L  I  Q  K  G  Q  T  Q  K  A  R  R  I  L  S  Q  M

CGTGGGAATC AGACCATTGA CGAGGAGTAT GACAGCATCA GGAACAGCAT CGAGGAGGAG  960
R  G  N  Q  T  I  D  E  E  Y  D  S  I  R  N  S  I  E  E  E

GAGAAGGAAG CCAGCGCAGC TGGACCTATA ATCTGCAGAA TGCTGAGTTA CCCCCCAACT 1020
E  K  E  A  S  A  A  G  P  I  I  C  R  M  L  S  Y  P  P  T

CGCCGAGCGT TAGCTGTGGG ATGTGGCTTA CAGATGTTCC AGCAGCTCTC GGGCATCAAC 1080
R  R  A  L  A  V  G  C  G  L  Q  M  F  Q  Q  L  S  G  I  N

ACTATCATGT ACTACAGCGC GACCATCCTG CAGATGTCCG GCGTGGAAGA TGATAGACTT 1140
T  I  M  Y  Y  S  A  T  I  L  Q  M  S  G  V  E  D  D  R  L

GCAATATGGC TGGCTTCCAT TACAGCGTTC ACCAATTTCA TTTTCACACT GGTGGGCGTC 1200
A  I  W  L  A  S  I  T  A  F  T  N  F  I  F  T  L  V  G  V

TGGCTCGTGG AGAAGGTGGG CCGCAGGAAG CTCACCTTTG GCAGTTTGGC AGGTACCACA 1260
W  L  V  E  K  V  G  R  R  K  L  T  F  G  S  L  A  G  T  T

GTAGCACTTA CAATTCTTGC CTTGGGATTT CTGCTCTCAG CTCAGGTCTC ACCACGGGTC 1320
V  A  L  T  I  L  A  L  G  F  L  L  S  A  Q  V  S  P  R  V

ACTTTCAGAC CAACGGCTCC CTCGGGTCAA AATGCCACCT GCACAGAATA CAGTTACTGT 1380
T  F  R  P  T  A  P  S  G  Q  N  A  T  C  T  E  Y  S  Y  C

AATGAGTGTA TGCTGGATCC AGACTGCGGT TTCTGCTACA AGATCAACAG TTCGGCTGTC 1440
N  E  C  M  L  D  P  D  C  G  F  C  Y  K  I  N  S  S  A  V

ATTGATTCCT CCTGTGTTCC GGTGAACAAA GCTTCCACCA ATGAAGCAGC CTGGGGCAGG 1500
I  D  S  S  C  V  P  V  N  K  A  S  T  N  E  A  A  W  G  R
```

```
TGTGAAAACG AAACCAAGTT CAAAGCAGAA GATGTTCACT GGGCTTACAG TTTCTGCCCT  1560
C  E  N  E  T  K  F  K  A  E  D  V  H  W  A  Y  S  F  C  P

ACCCCATACT CCTGGACAGC ACTCGTGGGC CTGGTGTTAT ATCTTGTTTT CTTTGCACCC  1620
T  P  Y  S  W  T  A  L  V  G  L  V  L  Y  L  V  F  F  A  P

GGAATGGGAC CAATGCCCTG GACCGTGAAC TCTGAAATCT ATCCTCTCTG GGCAAGAAGT  1680
G  M  G  P  M  P  W  T  V  N  S  E  I  Y  P  L  W  A  R  S

ACAGGAAATG CGTGCTCAGC TGGAATAAAC TGGATTTTCA ACGTGCTGGT TTCACTGACC  1740
T  G  N  A  C  S  A  G  I  N  W  I  F  N  V  L  V  S  L  T

TTTTTACACA CAGCGGAGTA TCTTACATAC TATGGAGCGT TCTTCCTCTA CGCCGGATTC  1800
F  L  H  T  A  E  Y  L  T  Y  Y  G  A  F  F  L  Y  A  G  F

GCCGCTGTGG GACTGCTTTT CGTCTATGGC TGTCTTCCTG AAACCAAAGG GAAAAAACTA  1860
A  A  V  G  L  L  F  V  Y  G  C  L  P  E  T  K  G  K  K  L

GAGGAAATCG AATCGCTCTT CGACCACCGG CTGTGCACCT GCGGCACCGC GGACTCGGAC  1920
E  E  I  E  S  L  F  D  H  R  L  C  T  C  G  T  A  D  S  D

GAGGGCAGGT ACATCGAGTA CATCCGCGTG AAGGGAAGTA ACTACCATCT CTCCGACAAC  1980
E  G  R  Y  I  E  Y  I  R  V  K  G  S  N  Y  H  L  S  D  N

GACGCCTCGG ACGTGGAGTG ACTTCCACCC GATGGCTTTC CAGTTACTGA AGTGAACGTG  2040
D  A  S  D  V  E

TCGGGAAGGC CAGCGGTTGG TCACTGCCCT GCTCACAAGC CGAGTCTCCC CACCGTCCTT  2100
GCCAATGACT CCATATTCCA GAAGACTTGA TGAGCAGAAA AATACAACTC TGGTGATGTT  2160
TATTTTCATA CACAGAAATG CTAAAAAAAA AAAATAAATA AATAACCACA GATTTTTCTT  2220
AGGCAAATGT GTACAAGCCC TTCCCGAGAC AATCTAAACA CTACTGTTGT ACCCAGCGAC  2280
TTCAGTTGGG GTCCCTTTCT CCTTAAACCA CACATAATTA GTGGCAATAC AGTCAGTGCT  2340
TACCTAGGCA GATTATTTAC GTATATTTAT AAAGGATTAT TTAAAACGAT CTAAGAATAT  2400
TTAATGTCAA AACACGGCCT ATTGACCCTA AATTTTCATA TCAGGTAGTG GCTTTTCTCT  2460
GATCAGTACA GAGTGAATCC CATCTAAACT TCCCCCTCGT GCCG                    2504
```

TABLE 4C

Sequence alignment of human and rat GLUTX2 protein sequences and Majority GLUTX2 Sequence (SEQ ID NO:16)

```
                                   oo
hX2       MGERRRKQPEPDAASAAGECSLLAAAESSTSLQSAGAGGGGVGDLERAARRQFQQDETPA
rX2       MGERRRRQPEP--GAPGGERSLLAA-ESAASLQGA--------ELERAARRQFQRDETPA
          ******:****  .:..** ***** **::***.*        :**********:*****
Majority  MGERRRkQPEPdaasaaGECSLLAAAESSTSLQSAgaggggvgdLERAARRQFQqDETPA

                 _________TM1_________                        _________TM2_______
hX2       FVYVVAVFSALGGFLFGYDTGVVSGAMLLLKRQLSLDALWQELLVSSTVGAAAVSALAGG
rX2       FVYAAAAFSALGGFLFGYDTGVVSGAMLLLRRQMRLGAMWQELLVSGAVGAAAVAALAGG
          ***..*.************************:**: *.*:********.:*******:*****
Majority  FVYvvAvFSALGGFLFGYDTGVVSGAMLLLkRQlsLdAlWQELLVSsTVGAAAVsALAGG

                          _______TM3_______                  ___________TM4_______
hX2       ALNGVFGRRAAILLASALFTAGSAVLAAANNKETLLAGRLVVGLGIGIASMTVPVYIAEV
rX2       ALNGALGRRSAILLASALCTVGSAVLAAAANKETLLAGRLVVGLGIGIASMTVPVYIAEV
          ****.:***:******** *.******** ****************************
Majority  ALNGvfGRRaAILLASALfTaGSAVLAAAnNKETLLAGRLVVGLGIGIASMTVPVYIAEV
```

```
                 _____TM5_____          _____TM6_____
hX2      SPPNLRGRLVTINTLFITGGQFFASVVDGAFSYLQKDGWRYMLGLAAVPAVIQFFGFLFL
rX2      SPPNLRGRLVTINTLFITGGQFFASVVDGAFSYLQKDGWRYMLGLAAIPAVIQFLGFLFL
         ************************************************:******:*****
Majority SPPNLRGRLVTINTLFITGGQFFASVVDGAFSYLQKDGWRYMLGLAAvPAVIQFfGFLFL

hX2      PESPRWLIQKGQTQKARRILSQMRGNQTIDEEYDSIKNNIEEEEKEVGSAGPVICRMLSY
rX2      PESPRWLIQKGQTQKARRILSQMRGNQTIDEEYDSIRNSIEEEEKEASAAGPIICRMLSY
         ************************************:*.*******..:***:*******
Majority PESPRWLIQKGQTQKARRILSQMRGNQTIDEEYDSIkNnIEEEEKEvgsAGPvICRMLSY

                 _____TM7_____                              _____TM8____
hX2      PPTRRALIVGCGLQMFQQLSGINTIMYYSATILQMSGVEDDRLAIWLASVTAFTNFIFTL
rX2      PPTRRALAVGCGLQMFQQLSGINTIMYYSATILQMSGVEDDRLAIWLASITAFTNFIFTL
         ******* ****************************************:**********
Majority PPTRRALiVGCGLQMFQQLSGINTIMYYSATILQMSGVEDDRLAIWLASvTAFTNFIFTL

                           _____TM9_____
hX2      VGVWLVEKVGRRKLTFGSLAGTTVALIILALGFVLSAQVSPRITFKPIAPSGQNATCTRY
rX2      VGVWLVEKVGRRKLTFGSLAGTTVALTILALGFLLSAQVSPRVTFRPTAPSGQNATCTEY
         ************************* ******:********:**:* **********.*
Majority VGVWLVEKVGRRKLTFGSLAGTTVALiILALGFvLSAQVSPRiTFkPiAPSGQNATCTrY

                           +
hX2      SYCNECMLDPDCGFCYKMNKSTVIDSSCVPVNKASTNEAAWGRCENETKFKTEDIFWAYN
rX2      SYCNECMLDPDCGFCYKINSSAVIDSSCVPVNKASTNEAAWGRCENETKFKAEDVHWAYS
         ****************:*.*:*******************************:**:.***.
Majority SYCNECMLDPDCGFCYKmNkStVIDSSCVPVNKASTNEAAWGRCENETKFKtEDifWAYn

                 _____TM10_____                    _____TM11____
hX2      FCPTPYSWTALLGLILYLVFFAPGMGPMPWTVNSEIYPLWARSTGNACSSGINWIFNVLV
rX2      FCPTPYSWTALVGLVLYLVFFAPGMGPMPWTVNSEIYPLWARSTGNACSAGINWIFNVLV
         ***********:**:*********************************:**********
Majority FCPTPYSWTALlGLiLYLVFFAPGMGPMPWTVNSEIYPLWARSTGNACSsGINWIFNVLV

         ____ _____TM12_____
hX2      SLTFLHTAEYLTYYGAFFLYAGFAAVGLLFIYGCLPETKGKKLEEIESLFDNRLCTCGTS
rX2      SLTFLHTAEYLTYYGAFFLYAGFAAVGLLFVYGCLPETKGKKLEEIESLFDHRLCTCGTA
         ****************************:*********************:*******:
Majority SLTFLHTAEYLTYYGAFFLYAGFAAVGLLFiYGCLPETKGKKLEEIESLFDnRLCTCGTs

hX2      DSDEGRYIEYIRVKGSNYHLSDNDASDVE
rX2      DSDEGRYIEYIRVKGSNYHLSDNDASDVE
         ****************************
Majority DSDEGRYIEYIRVKGSNYHLSDNDASDVE
```

Similar methodologies were used to clone human and rat GLUTX3, as shown in Tables 5A and 5B. An alignment of human (hX3) and rat (rX3) GLUTX3 is shown in Table 5C. The twelve putative TM domains are approximately delineated by the solid line above the aligned sequence. In Table 5C, fully conserved single residues are indicated by (*), fully conserved "strong" residues are indicated by (:), and fully conserved "weak" residues are indicated by (.). The putative glycosylated N residue is indicated by (+). The dileucine repeat is indicated by (o). The "Majority" GLUTX3 polypeptide sequence is disclosed as SEQ ID NO:17.

TABLE 5A

Human GLUTX3 Polynucleotide (SEQ ID NO:11) and Amino Acid (SEQ ID NO:12) Sequences

```
    1  gcccttggcc atgcaggagc cgctgctggg agccgagggc ccggactacg acaccttccc
                  M  Q  E  P  L  L  G  A  E  G  P  D  Y  D  T  F  P

   61  cgagaagccg cccccgtcgc caggggacag ggcgcgggtc gggaccctgc agaacaaaag
       E  K  P  P  P  S  P  G  D  R  A  R  V  G  T  L  Q  N  K  R

  121  ggtgttcctg gccaccttcg ccgcagtgct cggcaatttc agctttgggt atgccctggt
       V  F  L  A  T  F  A  A  V  L  G  N  F  S  F  G  Y  A  L  V

  181  ctacacatcc cctgtcatcc cagccctgga gcgctccttg gatcctgacc tgcatctgac
       Y  T  S  P  V  I  P  A  L  E  R  S  L  D  P  D  L  H  L  T

  241  caaatcccag gcatcctggt ttgggtccgt gttcaccctg ggagcagcgg ccggaggcct
       K  S  Q  A  S  W  F  G  S  V  F  T  L  G  A  A  A  G  G  L

  301  gagtgccatg atcctcaacg acctcctggg ccggaagctg agcatcatgt tctcagctgt
       S  A  M  I  L  N  D  L  L  G  R  K  L  S  I  M  F  S  A  V

  361  gccgtcggcg gccggctatg cggtcatggc gggtgcgcac ggcctctgga tgctgctgct
       P  S  A  A  G  Y  A  V  M  A  G  A  H  G  L  W  M  L  L  L

  421  cggaaggacg ctgacgggct tcgccggggg gctcacagct gcntgcatcc cggtgtacgt
       G  R  T  L  T  G  F  A  G  G  L  T  A  A  C  I  P  V  Y  V

  481  gtctgagatt gctcccccag gcgttcgtgg ggctctgggg gccacacccc agctcatggc
       S  E  I  A  P  P  G  V  R  G  A  L  G  A  T  P  Q  L  M  A

  541  agtgttcgga tccctgtccc tctacgccct tggcctcctg ctgccgtggc gctggctggc
       V  F  G  S  L  S  L  Y  A  L  G  L  L  L  P  W  R  W  L  A

  601  tgtggccggg naggcgcctg tgctcatcat gatcctgctg ctcagcttca tgcccaactc
       V  A  G  X  A  P  V  L  I  M  I  L  L  L  S  F  M  P  N  S

  661  gccgcgcttc ctgctctctc ggggcaggga cgaagaggcc ctgcgggcgc tggcctggct
       P  R  F  L  L  S  R  G  R  D  E  E  A  L  R  A  L  A  W  L

  721  gcgtgggacg gacgtcgatg tccactggga gttcgggcag atccaggaca acgtccggag
       R  G  T  D  V  D  V  H  W  E  F  G  Q  I  Q  D  N  V  R  R

  781  acagagcagc cgagtatcgt gggctgaggc acgggccccc cacgtgtgcc ggcccatcac
       Q  S  S  R  V  S  W  A  E  A  R  A  P  H  V  C  R  P  I  T

  841  cgtggccttg ctgatgcgcc tcctgcagca gctgacgggc atcacgccca tcctggtcta
       V  A  L  L  M  R  L  L  Q  Q  L  T  G  I  T  P  I  L  V  Y

  901  cctgcagtcc atcttcgaca gcaccgctgt cctgctgccc cccaaggacg acgcagccat
       L  Q  S  I  F  D  S  T  A  V  L  L  P  P  K  D  D  A  A  I

  961  cgttgggncc gtgcggctcc tgtccgtgct gatcgccgcc ctcaccatgg acctcgcagg
       V  G  X  V  R  L  L  S  V  L  I  A  A  L  T  M  D  L  A  G

 1021  ccgcaaggtg ctgctcttcg tctcagcggc catcatgttt gctgccaacc tgactctggg
       R  K  V  L  L  F  V  S  A  A  I  M  F  A  A  N  L  T  L  G

 1081  gctgtacatc cactttggcc ccaggcctct gagccccaac agcactgcgg gcctggaaag
       L  Y  I  H  F  G  P  R  P  L  S  P  N  S  T  A  G  L  E  S
```

```
1141  cgagtcctgg ggggacttgg cgcagcccct ggcagcaccc gctggctacc tcaccctggt
        E  S  W  G  D  L  A  Q  P  L  A  A  P  A  G  Y  L  T  L  V

1201  gcccctgctg gccaccatgc tcttcatcat gggctacgcc gtgggctggg gtcccatcac
        P  L  L  A  T  M  L  F  I  M  G  Y  A  V  G  W  G  P  I  T

1261  ctggctgctc atgtctgagg tcctaccccc gcgtgcccgt ggcgtggcct cagggctctg
        W  L  L  M  S  E  V  L  P  L  R  A  R  G  V  A  S  G  L  C

1321  cgtgctggcc agctggctca ccgccttcgt cctcaccaag tccttcctgc cagtggtgag
        V  L  A  S  W  L  T  A  F  V  L  T  K  S  F  L  P  V  V  S

1381  caccttcggc ctccaggtgc ctttcctctt cttcgcggcc atctgcctgg tgagcctggt
        T  F  G  L  Q  V  P  F  L  F  F  A  A  I  C  L  V  S  L  V

1441  gttcacaggc tgctgtgtgc ccgagaccaa gggacggtcc ctggagcaga tcgagtcctt
        F  T  G  C  C  V  P  E  T  K  G  R  S  L  E  Q  I  E  S  F

1501  cttccgcacg gggagaaggt ccttcttgcg ctagaagggc g
        F  R  T  G  R  R  S  F  L  R
```

TABLE 5B

Rat GLUTX3 Nucleotide (SEQ ID NO:13) and Amino Acid (SEQ ID NO:14) Sequences

```
ggcacgatgc aggagcccct gctaagagcc gagggactgg actatgacac cttccccgag  60
     M  Q  E  P  L  L  R  A  E  G  L  D  Y  D  T  F  P  E

gcgcccgcgt cgcctgaaga gaaggcacgg gccggggcct tgcaaaacag aagggtgttc 120
A  P  A  S  P  E  E  K  A  R  A  G  A  L  Q  N  R  R  V  F

ctggccacct tcgctgccgt gctgggcaat ttcagctttg ggtatgccct ggtctacaca 180
L  A  T  F  A  A  V  L  G  N  F  S  F  G  Y  A  L  V  Y  T

tctccagtca tccctgcgct gaagcgctct tctgacccag cactacgcct ggacaaaatc 240
S  P  V  I  P  A  L  K  R  S  S  D  P  A  L  R  L  D  K  I

caggcatcct ggtttgggtc cgtgttcacc ctgggtgccg ccgctggggg cctcagtgct 300
Q  A  S  W  F  G  S  V  F  T  L  G  A  A  A  G  G  L  S  A

atgctactca atgacctctt gggccggaag ctcagcatca tgttttccgc tgtcccctcg 360
M  L  L  N  D  L  L  G  R  K  L  S  I  M  F  S  A  V  P  S

gccattggct atgcactcat ggctggtgcc cgtggcctct ggatgcttct gctggggagg 420
A  I  G  Y  A  L  M  A  G  A  R  G  L  W  M  L  L  L  G  R

atgctgacag gctttgccgg gggactcact gctgcctgca tcccggtgta cgtgtctgag 480
M  L  T  G  F  A  G  G  L  T  A  A  C  I  P  V  Y  V  S  E

attgcacccc cgggtgttcg cggggccctg ggggccacgc cgcagctcat ggccgtgttt 540
I  A  P  P  G  V  R  G  A  L  G  A  T  P  Q  L  M  A  V  F

ggatccctgt ctctctatgc ccttggtctt ctgctgcctt ggcgatggct tgccgtggcc 600
G  S  L  S  L  Y  A  L  G  L  L  L  P  W  R  W  L  A  V  A

ggggaggggc ctgttctggt catgatcctg ctgcttagct tcatgcccaa ctcgcctcgc 660
G  E  G  P  V  L  V  M  I  L  L  L  S  F  M  P  N  S  P  R

ttcctactgt ctaagagccg ggatgaggaa gcactgcagg cgctgatctg gctgcgagcg 720
F  L  L  S  K  S  R  D  E  E  A  L  Q  A  L  I  W  L  R  A
```

```
gactctgagg tccactggga gtttgagcag atccaggaca acgtgcggag acagagtagc  780
D  S  E  V   H  W  E   F  E  Q   I  Q  D  N   V  R  R   Q  S  S

cgagtgtcgt gggcggaggc ctgggagccc cgtgtgtacc ggcctattct cattacagtg  840
R  V  S  W   A  E  A   W  E  P   R  V  Y  R   P  I  L   I  T  V

ctgatgcgtt ttctgcagca gctgacaggc atcactccca tcctcgtgta cctacagacc  900
L  M  R  F   L  Q  Q   L  T  G   I  T  P  I   L  V  Y   L  Q  T

atcttcgaca gcacgtccgt ggtgctgccc tcccagcagg atgcagctat agttggtgct  960
I  F  D  S   T  S  V   V  L  P   S  Q  Q  D   A  A  I   V  G  A

gtgaggctcc tgtctgtgct gattgccgct gtcaccatgg acctggccgg ccgaaaagtc 1020
V  R  L  L   S  V  L   I  A  A   V  T  M  D   L  A  G   R  K  V

ctgctctatg tgtcagcatc cattatgttt gtcgccaacc tgacgctggg gctgtatgtc 1080
L  L  Y  V   S  A  S   I  M  F   V  A  N  L   T  L  G   L  Y  V

cagcttgtgc caaggactct gacccccaac agcactgtgg agatcgtgac gcttgggggc 1140
Q  L  V  P   R  T  L   T  P  N   S  T  V  E   I  V  T   L  G  G

acggagcagc ccccagccgc ggccttcaac tatctcaccc tgataccccc gctggccacc 1200
T  E  Q  P   P  A  A   A  F  N   Y  L  T  L   I  P  L   L  A  T

atgctcttca ttatgggcta tgccatgggc tgggggccca tcacctggct cctcatgtct 1260
M  L  F  I   M  G  Y   A  M  G   W  G  P  I   T  W  L   L  M  S

gaggttctgc ccctgcgtgc ccgtggtgtg gcctcagggc tctgcgtgct ggtcagctgg 1320
E  V  L  P   L  R  A   R  G  V   A  S  G  L   C  V  L   V  S  W

ctcacagcct tcgtcctcac taagtacttc ctgctggcag tgaatgcctt cggcctccag 1380
L  T  A  F   V  L  T   K  Y  F   L  L  A  V   N  A  F   G  L  Q

gtgcctttct tcttcttctc ggccatctgc ctgctcagcc tgctcttcac aggctgctgt 1440
V  P  F  F   F  F  S   A  I  C   L  L  S  L   L  F  T   G  C  C

gtgcctgaga ccaggggccg ctcgctggag cagatcgagg ccttcttcca cactcgcagg 1500
V  P  E  T   R  G  R   S  L  E   Q  I  E  A   F  F  H   T  R  R

atgtccttca ggccctagtc aggggcttgc ctgtaagggg ccaggccatg tgggccaggc 1560
M  S  F  R   P  X

ctgtgcacac ctgcagccgg gagcagcagc acccctgccg tcagccactg cacacttcca 1620
cctsggacag gcagttcacc aactgtgggc tggtgggccc cagcaggcag cccgcagtgc 1680
tgtaatacct cagctgagat ccgcaggggg gcaggcagcc ctgccccaga ctgagcaaag 1740
gctgtaccca agaacaatgc tgttgggacc tccggtctag ttcctcattc ctacaaggaa 1800
cacttcccat gtagggccat gttgaggaca gaaaacaggg atggaggtgg caatgtggca 1860
ttgtacaagg acattgtcac ttccttctga tcaactggcc agggggagga cagggtcctg 1920
taccctcaag ggtctgggta ccttggggag cactggaccc agtccaacct tggatacctc 1980
tagtctacag actgttcact gtttttcaaa a                                2011
```

TABLE 5C

Sequence alignment of human and rat GLUTX3 protein sequences and Majority GLUTX3 Sequence (SEQ ID NO:17)

```
                  oo                                    TM1
hX3         MQEPLLGAEGPDYDTFPEKPPPSPGDRARVGTLQNKRVFLATFAAVLGNFSFGYALVYTS
rX3         MQEPLLRAEGLDYDTFPEAP-ASPEEKARAGALQNRRVFLATFAAVLGNFSFGYALVYTS
            ****** *** ******* * .** ::**.*:***:************************
Majority    MQEPLLgAEGpDYDTFPEkPpASPgdrARvGtLQNkRVFLATFAAVLGNFSFGYALVYTS
```

```
                                        TM2                 TM3
hX3         PVIPALERSLDPDLHLTKSQASWFGSVFTLGAAAGGLSAMILNDLLGRKLSIMFSAVPSA
rX3         PVIPALKRSSDPALRLDKIQASWFGSVFTLGAAAGGLSAMLLNDLLGRKLSIMFSAVPSA
            ******:** ** *:* * ********************:******************
Majority    PVIPALeRSlDPdLhLtKsQASWFGSVFTLGAAAGGLSAMiLNDLLGRKLSIMFSAVPSA

                      TM4                 TM5
hX3         AGYAVMAGAHGLWMLLLGRTLTGFAGGLTAACIPVYVSEIAPPGVRGALGATPQLMAVFG
rX3         IGYALMAGARGLWMLLLGRMLTGFAGGLTAACIPVYVSEIAPPGVRGALGATPQLMAVFG
             ***:****:********* ****************************************
Majority    aGYAvMAGAhGLWMLLLGRtLTGFAGGLTAACIPVYVSEIAPPGVRGALGATPQLMAVFG

                      TM6
hX3         SLSLYALGLLLPWRWLAVAGXAPVLIMILLLSFMPNSPRFLLSRGRDEEALRALAWLRGT
rX3         SLSLYALGLLLPWRWLAVAGEGPVLVMILLLSFMPNSPRFLLSKSRDEEALQALIWLR-A
            ******************* .***:*****************:.******:** *** :
Majority    SLSLYALGLLLPWRWLAVAGXaPVLiMILLLSFMPNSPRFLLSrgRDEEALrALaWLRgt

                                                TM7
hX3         DVDVHWEFGQIQDNVRRQSSRVSWAEARAPHVCRPITVALLMRLLQQLTGITPILVYLQS
rX3         DSEVHWEFEQIQDNVRRQSSRVSWAEAWEPRVYRPILITVLMRFLQQLTGITPILVYLQT
            * :***** ****************  *:* *** :::***:***************:
Majority    DvdVHWEFgQIQDNVRRQSSRVSWAEAraPhVcRPItvalLMRlLQQLTGITPILVYLQs

                      TM8                 TM9
hX3         IFDSTAVLLPPKDDAAIVGXVRLLSVLIAALTMDLAGRKVLLFVSAAIMFAANLTLGLYI
rX3         IFDSTSVVLPSQQDAAIVGAVRLLSVLIAAVTMDLAGRKVLLYVSASIMFVANLTLGLYV
            *****:*:**.::****** **********:***********:***:***.********:
Majority    IFDSTaVlLPPkdDAAIVGaVRLLSVLIAAlTMDLAGRKVLLfVSAaIMFaANLTLGLYi

                  +                                 TM10
hX3         HFGPRPLSPNSTAGLESESWGDLAQPLAAPAGYLTLVPLLATMLFIMGYAVGWGPITWLL
rX3         QLVPRTLTPNSTVEIVT--LGGTEQPPAAAFNYLTLIPLLATMLFIMGYAMGWGPITWLL
            :: **.*:****. : :   *.  ** **. .****:**************:*********
Majority    hfgPRrLsPNSTagleseswGdlaQPlAApagYLTLvPLLATMLFIMGYAvGWGPITWLL

                      TM11                TM12
hX3         MSEVLPLRARGVASGLCVLASWLTAFVLTKSFLPVVSTFGLQVPFLFFAAICLVSLVFTG
rX3         MSEVLPLRARGVASGLCVLVSWLTAFVLTKYFLLAVNAFGLQVPFFFFSAICLLSLLFTG
            ******************.********** ** .*.:********:**:****:**:***
Majority    MSEVLPLRARGVASGLCVLaSWLTAFVLTKsFLpvVstFGLQVPFlFFaAICLvSLvFTG

hX3         CCVPETKGRSLEQIESFFRTGRRSFLR
rX3         CCVPETRGRSLEQIEAFFHTRRMSFRP
            ******:********:**:* * **
Majority    CCVPETkGRSLEQIEsFFrTgRrSFlr
```

The GLUTX family members were aligned using ClustalX and ClustalW software analyses. The alignment of human GLUTX1, GLUTX2 and GLUTX3 protein sequences and the human GLUTX consensus sequence (SEQ ID NO:18) are shown in Tables 6E and 6A, respectively. The alignment of rat GLUTX1, GLUTX2 and GLUTX3 protein sequences is shown in FIG. 3, with alternative alignments indicated in Tables 6C and 6F and described below. The rat GLUTX consensus sequence from FIG. 3 (SEQ ID NO:19) is shown in Table 6B. Underlined sequences in the various tables depicting consensus sequences, *i.e.*, Tables

6A, 6B and 6D, depict residues that are conserved between the GLUTX family of sequences but are not conserved between the GLUTX family and the GLUT1-5 family of proteins. Conserved regions between GLUTX1 and the GLUT family of sequences are indicated by dark and shaded residues in FIG. 1 and described more fully in the DETAILED DESCRIPTION above. GLUTX2 and GLUTX3 homologies to the GLUT family of sequences can easily be determined by one skilled in the art by the juxtaposing of the alignments of GLUTX1-3 in the tables below with the sequence of GLUTX1, and hence with the other GLUT family member, in FIG. 1.

Table 6C represents an alternative alignment of GLUTX1-3 residues around the single putative glycosylation site, as compared to the alignment in FIG. 3. In Table 6C, the putative glycosylated N residue, indicated by (+), is aligned between all three polypeptides. The modified alignment creates an alternative rat GLUTX consensus sequence (SEQ ID NO:20) for the surrounding region, as shown in Table 6D. An alignment of human GLUTX1-3 using ClustalW (Table 6E) was used to generate a "Majority" human GLUTX sequence (SEQ ID NO:21). Alignments rat GLUTX proteins were determined with ClustalW version 1.7 software (Table 6F) using default parameters, and with ClustalX software (FIG. 2) as described above. The alternative alignment of rat GLUTX1-3 using ClustalW (Table 6F) was used to generate a "Majority" rat GLUTX sequence (SEQ ID NO:22) depicting the most common residues at a given position in the alignment. An "x" at any given position denotes that all residues at that position are different in each of the GLUTX sequences in the alignment. A residue designated by an "x" may be strongly conserved, weakly conserved, or nonconserved. The degree of conservation will be indicated in its respective table.

In the consensus sequences for human and rat GLUTX, shown in Tables 6A, 6B and 6D and provided as SEQ ID NOs:18, 19 and 20, upper case letters denote a fully conserved single residue that appears in the same position in all three GLUTX sequences. Lower case letters denote conserved residues that appear in the same position in two of the three GLUTX sequences used in the alignment. Shaded regions in the consensus sequences indicate residues that appear only in GLUTX2 (x), only in GLUTX1 and GLUTX3 but not GLUTX2 (-x-), and only in GLUTX2 and GLUTX3 but not GLUTX1 (-x-). In alternative embodiments, polypeptides representing GLUTX consensus sequence may or may not contain the residues in one or more of the regions indicated by shading. For example, all or some of the GLUTX2 specific residues may be omitted from a consensus polypeptide. Alternatively, all or some of the residues specific to GLUTX2 and GLUTX3, but not in GLUTX1, may be omitted from a

consensus polypeptide. Nucleic acids encoding the shaded residues may likewise be omitted in alternative embodiment of GLUTX consensus nucleotide sequences.

TABLE 6A

Consensus Human GLUTX Amino Acid (SEQ ID NO:18) Sequence from FIG. 3A

```
KEY: GLUTX2 specific = -x-
     GLUTX 1+3 only  = -x-
     GLUTX 2+3 only  = -x-
     * = variable number of N-ter residues after the M translation start
site
     - = spacers for consensus residues indicating variable
     Upper case = identical residues
     Lower case = conserved strong and weak residues

Mgerrr--pEpdaasa-----LL-A-gp--------pggs----a-R--Rr---Q---E-   60

EVflaafaaaLG-fsFFyalgy-Spaip-L-R-l-P---Ld---aswfgsv-tlgAAagg  120

l-gg-Lnd--Grklsill-svpf-AG-AV-aaA---wmLL-GrlltGla-G-as---PVY  180

isEiapP-vRG-Lg---qlm-v-G---ay--g--------l-Wrwlavlg-vP--im-ll  240

--PmPesPRfLl--gr-geA-raL--lrG------wE---I-dn----e--v--A-----  300

E-lr-P---rp-ivg--l--fQQLsGin-im-Y--tIf----v----------a-vvg--  360

--lftlvaal-md-aGRr-Llf-S-a-m--a---lglyf-l----------P--E-     420

----------------------------------s-s-pv-aqp--a-ag----------  480

----------l-w-all---Lfi-gfAvGwGPipWllmSEi-PL-argva-g-cvl-nW  540

l-aflvtk-Fl---e-l--ygaF-l-aafci-sllFt--CvPEtKGk-LeqIes-F---R  600

-----------------------------------------------------------  636
```

TABLE 6B

Consensus Rat GLUTX Amino Acid (SEQ ID NO:19) Sequence from FIG. 3B

```
KEY: GLUTX2 specific = -x-
     GLUTX 1+3 only  = -x-
     GLUTX 2+3 only  = -x-
     * = variable number of N-ter residues after the M translation start
site
     - = spacers for consensus residues indicating variable
     Upper case = identical residues
     Lower case = conserved strong and weak residues

          *
    1  M-pE--dp-----llasp-ara-gGa---E-RR----------vflAtf

   50  aaaLG-fsFGyalgysSpaip-LrRt--palRLg---AsWfg-vvt-E-lG

  100  AAA-ggl-gg-Lnd-lGRklsill-tvp--iGfaviaaAr-vwmLLgGRl
```

```
150  ltGla-Gvasl-iPVYisEiapPavRG-Lgs--qLmvv-G-l-ayvlg
200  wll-WRwlavlg-vP-llmlLlm-fmPesPRfLltk-q-geAm-aL
250  -flrg-sev-wEfe-l---vehq----s-Aml--P-vhrpliig
300  i-l-vfQQLsGin-ImfY-nTIfe-t-v-d-s---a-ivg-iqvlftl
350  vaalimdraGRklLly-lsgsim-vsmlalG-ly-qltpr--gP
400  snss-vv-tlvpi-qepA
450  dvn-g-ltwlallammLfimgfAvGwGPipW
500  llmSEifPLhargvatG-vCvl-vnWl-aflvtk-Fl--me-l--ygaFf
550  lfaafcilslLFt--cvPETkGrtLEqlea-Fe-R--s---
600
```

TABLE 6C

Partial Alternative Alignment for Consensus Rat GLUTX Amino Acid Sequences in FIG. 3

```
                                                                +
rGLUTx1_pep    346  S------GPS---------------------------NSSHVG--LLV
rGLUTx3_pep    346  RT----LTP---------------------------NSTVEIV-TLG
rGLUTx2_pep    346  RVTFRPTAPSGQNATCTEYSYCNECMLDPDCGFCYKINSSAVIDSSCV
consensus      421  r------gPS---------------------------NSs-vi--tlv
```

TABLE 6D

Alternative Consensus Rat GLUTX Amino Acid (SEQ ID NO:20) Sequence

```
KEY: GLUTX2 specific = -x-
     GLUTX 1+3 only  = -x-
     GLUTX 2+3 only  = -x-
     * = variable number of N-ter residues after the M translation start
site
     - = spacers for consensus residues indicating variable
     Upper case = identical residues
     Lower case = conserved strong and weak residues

        *
   1  M-pE--dp-----llasp-ara-gGa---RRvflAtf
  50  aaaLG-fsFGyalgysSpaip-LrRt--palRLg---AsWfg-vvt-lG
 100  AAA-ggl-gg-Lnd-lGRklsill-tvp--iGfaviaaAr-vwmLLgGRl
 150  ltGla-Gvasl-iPVYisEiapPavRG-Lgs--qLmvv-G-l-ayvlg-
 200  -wll--WRwlavlg-vP-llmlLlm-fmPesPRfLltk-q-geAm-aL
 250  -flrg-sev-wEfe-l---vehq----s-Aml--P-vhrpliig
 300  i-l-vfQQLsGin-ImfY-nTIfe-t-v-d--s---a-ivg-iqvlftl
 350  vaalimdraGRklLly-lsgsim-vsmlalG-ly-qltpr--gP
 400  SNSs-vi-tlvpi-qepA
```

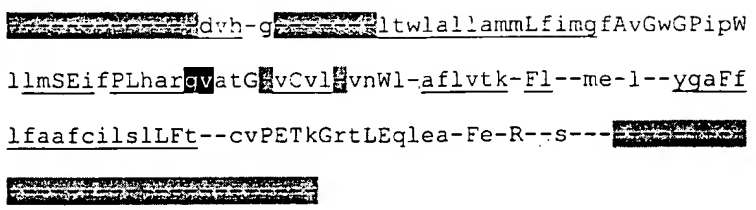


TABLE 6E

Sequence alignment of human GLUTX1, GLUTX2 and GLUTX3 polypeptide sequences and Majority human GLUTX Sequence (SEQ ID NO:21)

```
hX3       -------MQEP----------LLGAEGPDYDTFPEKPPPSPGDRA----RVGTLQNKR--
hX1       ------MTPEDP----EETQPLL---GP--------PGGS----APRG-RR---------
hX2       MGERRRKQPEPDAASAAGECSLLAAAESSTSLQSAGAGGGGVGDLERAARRQFQQDETPA
                    *           .**.*  ....  .  .  .  .   *  *    *:.
Majority  MgerrrxxpepdaasaxxxxxllxAxgpxxxxxxxxpggsxxxxaxRxaRrxxxQxxxpa

hX3       -VFLATFAAVLGNFSFGYALVYTSPVIPALERSLDPDLHLTKSQASWFGSVFTLGAAAGG
hX1       -VFLAAFAAALGPLSFGFALGYSSPAIPSLQRAAPPAPRLDDAAASWFGAVVTLGAAAGG
hX2       FVYVVAVFSALGGFLFGYDTGVVSGAMLLLKRQLS----LDALWQELLVSSTVGAAAVSA
           *:::.:. :.** : **:     * .:  *:*       *     . : :  . .**...
Majority  fVflaafaaaLGxfsFFyalgyxSpaipxLxRxlxPxxxLdxxxaswfgsvxtlgAAagg

hX3       LSAMILNDLLGRKLSIMFSAVPSAAGYAVMAGAHGLWMLLLGRTLTGFAGGLTAACIPVY
hX1       VLGGWLVDRAGRKLSLLLCSVPFVAGFAVITAAQDVWMLLGGRLLTGLACGVASLVAPVY
hX2       LAGGALNGVFGRRAAILLASALFTAGSAVLAAANNKETLLAGRLVVGLGIGIASMTVPVY
          : .  * .  **: ::::.:.  .** **::.*:.   ** ** :.*:. *:::   ***
Majority  lxggxLndxxGrklsillxsvpfxAGxAVxaaAxxxwmLLxGrlltGlaxGxasxxxPVY

hX3       VSEIAPPGVRGALGATPQLMAVFGSLSLYALG------LLLPWRWLAVAGXAPVLIMILL
hX1       ISEIAYPAVRGLLGSCVQLMVVVGILLAYLAG------WVLEWRWLAVLGCVPPSLMLLL
hX2       IAEVSPPNLRGRLVTINTLFITGGQFFASVVDGAFSYLQKDGWRYMLGLAAVPAVIQFFG
          ::*:: * :** * :   *: . * :     .          **::   . .*  : ::
Majority  isEiappPxvRGxLgxxxqlmxvxGxxxayxxggafsylxxlxWrwlavlgxvPxximxll

hX3       LSFMPNSPRFLLSRGRDEEALRALAWLRGTDVDVHWEFGQIQDNVRRQSSRVSWA-----
hX1       MCFMPETPRFLLTQHRRQEAMAALRFLWGSEQ--GWE-----DPPIGAEQSFHLA-----
hX2       FLFLPESPRWLIQKGQTQKARRILSQMRGNQT-IDEEYDSIKNNIEEEEKEVGSAGPVIC
          : *:*::**:*: : : ::*   *  : *.:     *     :     .. .  *
Majority  xxFmPesPRfLlxxgrxqeAxraLxxlrGxxxdxxwExxxIxdnxxxxexxvxxAgpvic

hX3       -EARAPHVCRPITVALLMRLLQQLTGITPILVYLQSIFDSTAVLLP---PKDDAAIVGXV
hX1       -LLRQPGIYKPFIIGVSLMAFQQLSGVNAVMFYAETIFEEAKFKDS----SLASVVVGVI
hX2       RMLSYPPTRRALIVGCGLQMFQQLSGINTIMYYSATILQMSGVEDDRLA-IWLASVTAFT
               *   :.: :.  :  :***:*:..:: *  :*:: : .          : :..
Majority  rxlrxPxxxrpxivgxxlxxfQQLsGinximxYxxtIfxxxxvxxxrlapxxxaxvvgxx

hX3       RLLSVLIAALTMDLAGRKVLLFVSAAIMFAANLTLGLYIHFGPRP---------LSPNS--
hX1       QVLFTAVAALIMDRAGRRLLLVLSGVVMVFSTSAFGAYFKLTQG----------GPG---
hX2       NFIFTLVGVWLVEKVGRRKLTFGSLAGTTVALIILALGFVLSAQVSPRITFKPIAPSGQN
          ..: . :..  :: .**: * . * .    :   :.  : :            .*.
Majority  xxlftlvaalxmdxaGRrxLlfxSxaxmxxaxxxlglyfxlxxxxspritfkpxxPxxqn

hX3       -------------------------TAGLESESWGDLAQPLAAPAGY-----------
hX1       -------------------------NSSHVAISAPVSAQPVDASVG-------------
hX2       ATCTRYSYCNECMLDPDCGFCYKMNKSTVIDSSCVPVNKASTNEAAWGRCENETKFKTED
                                  .:   : .      .   ..
Majority  atctrysycnecmldpdcgfcykmnkxxxxxsxsxpvxaqpxxaxagxrcemetlflted
```

```
hX3       -----------LTLVPLLATMLFIMGYAVGWGPITWLLMSEVLPLRARGVASGLCVLASW
hX1       -----------LAWLAVGNMCLFIAGFAVGWGPIPWLLMSEIFPLHVKGVATGICVLTNW
hX2       IFWAYNFCPTPYSWTALLGLILYLVFFAPGMGPMPWTVNSEIYPLWARSTGNACSSGINW
                     :  .:    *::  :* * **:.* : **: ** .:..... .   .*
Majority  ifwaynfcptplxwxallxxxLfixgfAvGwGPipWllmSEixPLxargvaxgxcvlxnW

hX3       LTAFVLTKSFLPVVSTFGLQVPFLFFAAICLVSLVFTGCCVPETKGRSLEQIESFFRTGR
hX1       LMAFLVTKEFSSLMEVLRPYGAFWLASAFCIFSVLFTLFCVPEIKGKTLEQITAHFEG-R
hX2       IFNVLVSLTFLHTAEYLTYYGAFFLYAGFAAVGLLFIYGCLPETKGKKLEEIESLFDN-R
          :  .::::  *    . :    .* : :.:. ..::*   *:** **:.**:* : *   *
Majority  lxaflvtkxFlxxxexlxxygaFxlxaafcixsllFtxxCvPEtKGkxLeqIesxFxxgR

hX3       RSFLR-------------------------------
hX1       -----------------------------------
hX2       LCTCGTSDSDEGRYIEYIRVKGSNYHLSDNDASDVE
Majority  xxxxxtsdsdegryieyirvkgsnyhlsdndasdve
```

TABLE 6F

Alternative Sequence alignment of rat GLUTX1, GLUTX2 and GLUTX3 protein sequences and Majority rat GLUTX Sequence (SEQ ID NO:22)

```
glutx3rat       -----------MQEPLLRAEGLDYDTFPEAPASPEEKARAGALQN---RRVFLATFAAVL
glutx1rat       ------------------------MSPEDPQETQPLLRSPGARAPGGRRVFLATFAAAL
glutx2rat       MGERRRRQPEPGAPGGERSLLAAESAASLQGAELERAARRQFQRDETPAFVYAAAAFSAL
                                        .    . :   *    :      *: *:  :.*
Majority        mgerrrrqpepxxxxxxRxxxxxxxxxpexpaexexxaRxxxxrxxxxrrVflAtfaaaL

glutx3rat       GNFSFGYALVYTSPVIPALKRSSDPALRLDKIQASWFGSVFTLGAAAGGLSAMLLNDLLG
glutx1rat       GPLSFGFALGYSSPAIPSLRRTAPPALRLGDTAASWFGAVVTLGAAAGGVLGGWLLDRAG
glutx2rat       GGFLFGYDTGVVSGAMLLLRR----QMRLGAMWQELLVSGAVGAAAVAALAGGALNGALG
                * : **:     * .:  *:*     :**.    . : :  . .**...: .  * .  *
Majority        GxfsFGyalgyxSpxipxLrRxxxxalRLgxxxaswfgsvxtlgAAagglxggxLndxlG

glutx3rat       RKLSIMFSAVPSAIGYALMAGARGLWMLLLGRMLTGFAGGLTAACIPVYVSEIAPPGVRG
glutx1rat       RKLSLLLCTVPFVTGFAVITAARDVWMLLGGRLLTGLACGVASLVAPVYISEIAYPAVRG
glutx2rat       RRSAILLASALCTVGSAVLAAAANKETLLAGRLVVGLGIGIASMTVPVYIAEVSPPNLRG
                *: :::::.:.  . * *:::.* .   ** **::.*:. *:::   ***::*:: * :**
Majority        RklsillxxvpxxxGxAvxaaarxxwmLLxGRlltGlaxGxasxxxPVYisSiapPxvRG

glutx3rat       ALGATPQLMAVFGSLSLYALG------LLLPWRWLAVAGEGPVLVMILLLSFMPNSPRFL
glutx1rat       LLGSCVQLMVVTGILLAYVAG------WVLEWRWLAVLGCVPPTLMLLLMCYMPETPRFL
glutx2rat       RLVTINTLFITGGQFFASVVDGAFSYLQKDGWRYMLGLAAIPAVIQFLGFLFLPESPRWL
                 * :   *: . * :   . .          **::   .  *  : :* : ::*::**:*
Majority        xLgxxxqLmxvxGxlxayvxggafsylxxlxWRwlavlgxxPxxxmlLlxxfmPesPRfL

glutx3rat       LSKSRDEEALQALIWLRADSEVHWEFEQIQDNVRRQSS------RVSWAEAWEPRVYRPI
glutx1rat       LTQHQYQEAMAALRFLWG-SEEGWE----EPPVGAEHQ------GFQLAMLRRPGVHKPL
glutx2rat       IQKGQTQKARRILSQMRGNQTIDEEYDSIRNSIEEEEKEASAAGPIICRMLSYPPTRRAL
                : : : ::*   *  : . .   *    .  : : .       .        * . :.:
Majority        lxkxqxqeAxxaLxxlrgxsexxwExxxixxxvxxxxxeasaagxxxxamlxxPxxxrpl

glutx3rat       LITVLMRFLQQLTGITPILVYLQTIFDSTSVVLP--SQQDAAIVGAVRLLSVLIAAVTMD
glutx1rat       IIGICLMVFQQLSGVNAIMFYANTIFEEAKFKD---SSLASVTVGIIQVLFTAVAALIMD
glutx2rat       AVGCGLQMFQQLSGINTIMYYSATILQMSGVEDDRLAIWLASITAFTNFIFTLVGVWLVE
                 :   : .:***:*:..*: *  **:: : .     :   :  ..  ..: . :..  ::
Majority        xigxxlxxfQQLsGinxImxYxxTIfxxxxxxdxrlsxxxaxivgxxxxlftlvaaxxmd
```

```
glutx3rat       LAGRKVLLYVSASIMFVANLTLGLYVQLVPRT--------LTPNST--------------
glutx1rat       RAGRKLLLALSGVIMVFSMSAFGTYFKLTQS----------GPSNS--------------
glutx2rat       KVGRRKLTFGSLAGTTVALTILALGFLLSAQVSPRVTFRPTAPSGQNATCTEYSYCNECM
                 .**: *   *     .:   :.  . *               *..
Majority        xaGRkxLlxxSxximxvaxxxxgxyfxLxxxxsprvtfrpxxPsxxnatcteysycnecm

glutx3rat       ----------VEIVTLGGTEQPPAAAFNY----------------------------LT
glutx1rat       ----------SHVGLLVPISAEPADVHLG----------------------------LA
glutx2rat       LDPDCGFCYKINSSAVIDSSCVPVNKASTNEAAWGRCENETKFKAEDVHWAYSFCPTPYS
                           .   :   .  *.                                  :
Majority        ldpdcgfcykxxxxxlxxxsxxPaxxxxxneaawgrcenetkfkaedvhwaysfcptplx

glutx3rat       LIPLLATMLFIMGYAMGWGPITWLLMSEVLPLRARGVASGLCVLVSWLTAFVLTKYFLLA
glutx1rat       WLAVGSMCLFIAGFAVGWGPIPWLLMSEIFPLHIKGVATGVCVLTNWFMAFLVTKEFNSI
glutx2rat       WTALVGLVLYLVFFAPGMGPMPWTVNSEIYPLWARSTGNACSAGINWIFNVLVSLTFLHT
                  .: .  *::  :* * **:.* : **: **  :..... ..  .*:  .:::  *
Majority        wlalxxxxLfixgfAxGwGPipWllmSEixPLxargvaxgxcvlxnWxxaflvtkxFlxx

glutx3rat       VNAFGLQVPFFFFSAICLLSLLFTGCCVPETRGRSLEQIEAFFHTRRMSFRP--------
glutx1rat       MEILRPYGAFWLTAAFCILSVLFTLTFVPETKGRTLEQITAHFEGR--------------
glutx2rat       AEYLTYYGAFFLYAGFAAVGLLFVYGCLPETKGKKLEEIESLFDHRLCTCGTADSDEGRY
                 : :    .*:: :.:. :.:**.   :***:*:.**:* : *. *
Majority        xexlxxygaFflxaafcilslLFtxxcvPETkGrxLEqIeaxFxxRxxxxxxadsdegry

glutx3rat       ----------------------
glutx1rat       ----------------------
glutx2rat       IEYIRVKGSNYHLSDNDASDVE
Majority        ieyirvkgsnyhlsdndasdve
```

Example 2. Northern Blot Analysis

GLUTX1 tissue distribution was assessed by Northern blot analysis of adult rat tissues total RNA. The strongest expression was seen in testis, with moderate expression in the cerebellum, brain stem, hippocampus, hypothalamus, adrenal gland, liver, spleen, brown adipose tissue, and lung. A low level of expression was detected in white adipose tissue, muscle, kidney, tongue and was barely detectable in intestine and stomach. No GLUTX1 expression was detected in thyroid.

Transient expression of both GLUTX2 and GLUTX3 transporters in HEK293 cells and immunofluorescence microscopic detection revealed that both GLUTX2 and GLUTX3 were located in intracellular compartment. For GLUTX2, at least, surface expression could be induced by mutating a dileucine internalization motif present the amino-terminal tail of the transporter and a tyrosine-based motif present in the carboxy-terininal cytoplasmic tail. GLUTX3 also possesses dileucine and tyrosine-based internalization motifs.

The tissue localization of GLUTX2 and GLUTX3 almost exclusively in the brain, their intracellular localization and the presence of dileucine motifs suggest the surface expression of these molecules may be triggered by some as yet unknown stimuli which may be coupled with increased brain activity. It has indeed been shown that stimulation of activity of different

brain regions is associated by increased glucose metabolism, that ischemia leads to increase glucose utilization and that diseases of the central nervous system may be associated with impaired stimulation of glucose uptake by specific brain areas

Methods: Total RNA was isolated as described. See, *e.g.*, Chomczynski and Sacchi 1987 *Anal. Biochem.* 162: 156-159. Northern blot analysis was with 10 μg of total RNA, and detection was with a 1.2-kilobase *Not*I-*Bgl*II fragment from the coding sequence of GLUTX1 radioactively labeled by random priming.

Example 3. GLUTX Protein Analysis

GLUTX1 synthetic mRNA was *in vitro* translated in reticulocyte lysate in the presence or absence of canine pancreas microsomal membranes. Gel electrophoresis results showed that the primary translation product migrated as a 35-kDa band, a higher electrophoretic mobility as compared with the predicted molecular mass of 51 kDa. In the presence of microsomal membranes, a part of the 35-kDa band was converted to a higher molecular mass of 37 kDa. This was due to *N*-glycosylation of the protein as demonstrated by the susceptibility of this band to digestion by both endoglycosaminidase H and PNGaseF. This modification probably takes place at the unique *N*-glycosylation site present in the exoplasmic loop between TM9 and TM10.

An *in vitro* translation of GLUTX1 cRNA using rabbit reticulocyte lysate in the presence (*+MM*) or absence (*MM*) of canine pancreatic microsomal membranes is described in the Method section below. In the reaction without microsomal membranes, only one band of approximately 35 kDa is present. An additional band of approximately 37 kDa is present in the reaction with microsomal membranes, indicating that this is the glycosylated form of GLUTX1. This was confirmed by sensitivity of the 37-kDa band to EndoHf or PNGaseF. *B*, Western blot of membrane proteins from HEK293T cells transiently transfected with wild-type GLUTX1 and control 293T cells. Membrane protein preparations from transfected cells were incubated at 37 °C for 2 h in the presence or absence of PNGaseF as described under "Materials and Methods." Each lane contains 20 μg of protein. Immunodetection was with the affinity purified GLUTX1(203-257) antibody as described below.

Methods: *In vitro* transcription was performed from linearized pSD5-GLUTX1 vector using SP6 RNA polymerase (Promega). cRNA was *in vitro* translated in rabbit reticulocyte lysate in the presence or absence of canine microsomal membranes (Promega) and [^{35}S]methionine (NEN Life Science Products). Products were separate on 10% polyacrylamide gels and detected by autoradiography.

For Western blotting, GLUTX1 was transiently transfected in HEK293T cells, and cell membranes were prepared and analyzed using specific antibodies following previously published procedures. See, *e.g.*, Jordan *et al.* 1996 *Nucleic Acids Res.* 24: 596-601; Widmann *et al.* 1995 *Biochem. J.* 310: 203-214. Endoglycosidase H and PNGaseF (New England Biolabs) digestions were performed according to the manufacturer's protocol.

Immunofluorescence microscopic detection of GLUTX1 or GLUTX1(LL-AA) was performed following transient transfection of HEK293T cells as previously described using 10 µg/ml GLUTX1 affinity-purified antibody and CY3-conjugated goat anti-rabbit antibody (Jackson ImmunoResearch). See, *e.g.*, Gilboe *et al.* 1972 *Anal. Biochem.* 47: 20-27.

Example 4. Functional Characterization of GLUTX in Xenopus Oocytes

To determine whether GLUTX1 was a functional glucose transporter, synthetic mRNAs were transcribed *in vitro* from the full-length rat cDNA and injected in *Xenopus* oocytes, and 2-DOG uptake was measured 3 days later. As shown in FIG. 5, 2-DOG uptake by oocytes injected with the wild-type GLUTX1 mRNA was not significant. We suspected that the lack of transport activity was due to the dileucine motif present at the amino-terminal end of the protein, which could serve as an internalization signal preventing sufficient surface expression of GLUTX1. We therefore mutated these two leucines into alanines and injected oocytes with the corresponding mutant mRNA. Transport activity by these injected oocytes was now strongly stimulated (FIG. 5 Panel A).

FIG. 5 Panel B shows that a 100-fold excess of D-glucose could completely block radioactive 2-DOG uptake, whereas L-glucose was not a competitor. D-Fructose and D-galactose could also inhibit glucose uptake, although with a reduced efficacy as compared with D-glucose. Uptake could also be blocked by cytochalasin B, a specific inhibitor of all the mammalian glucose transporter isoforms. Finally, the affinity of the transporter for glucose was determined, as shown in FIG. 5 Panel C. Michaelis-Menten analysis of the fitted curve indicates a K_m for 2-DOG of approximately 2.4 mM.

Analysis of GLUTX2 is shown in FIG. 6. Methods are discussed below.

Methods: Stage V-VI oocytes were injected with 25 ng of RNA prepared from GLUTX1 and GLUTX1(LL-AA) cDNA cloned into pSD5, as described. See, *e.g.*, Geering *et al.* 1996 *J. Cell Biol.* 133: 1193-1204. 2-Deoxy-D-glucose (2-DOG) uptake assays were performed 3 days following injection with groups of 8-10 oocytes incubated in the presence of 2 mM 2-DOG and 20 µCi of [1,2-^{3}H]deoxy-D-glucose (Moravek Biochemicals) for 45 min. 2-DOG uptake was previously confirmed to be linear with time throughout this period.

Oocytes were then washed with ice-cold solution containing 1 mM $HgCl_2$. Individual oocytes were dissolved in 2% SDS, mixed with scintillation fluid, and counted. For determination of the Michaelis-Menten constant, uptake was for 15 min at the indicated 2-DOG concentration.

In FIG. 5, panel A, 2-DOG uptake was measured in stage V-VI *Xenopus* oocytes injected with 25 ng of GLUTX1 wild-type (*WT*) RNA and GLUTX1(LL-AA) RNA compared with noninjected control oocytes. The concentration of 2-DOG was 2 mM. Each bar represents the average for 10 oocytes. In FIG. 5, panel B, all conditions used 2-DOG concentration of 2 mM. 8-10 oocytes per condition were incubated with 250 mM D-glucose, 250 mM L-glucose, 250 mM D-galactose, 250 mM D-fructose, or 50 μM cytochalasin B. Results are expressed as percentages of average 2-DOG uptake in nontreated oocytes (*top bar*). The results shown are an average of two experiments performed with two different batches of oocytes. In FIG. 5, panel C, 2-DOG uptake was measured at varying concentrations from 0.07 to 10 mM. Each point represents the average uptake from 8 to 10 oocytes. Fig 6 shows the dose curve for uptake of myo-inositol in oocytes injected with 25 ng of GlutX2(RRR-AAA) RNA, fitted using the Michaelis-Menten equation. Myo-inositol uptake was measured at varying concentration from 2.5 microM to 5 mM. Each point represents the average uptake from 8 to 10 oocytes.

Example 5. GLUTX Antibodies

To further characterize the protein, antibodies were raised against either the middle intracellular loop of the transporter or against its carboxyl-terminal tail. FIG. 4 shows the rat GLUTX amino acid sequence with position of the transmembrane domains, N-linked glycosylation site, LL internalization motif and the sequence of the proteins that were used for generating antibodies. Boxes indicate the putative TM domains. The underlined sequences are the sequences used to construct fusion proteins with GST for antibody production in rabbits. The LL motif in the amino-terminal domain, when mutated to AA (*i.e.*, Ala-Ala), allows surface expression of the GLUTX transporter protein. This GLUTX(LL) mutant was used to analyze expression in oocytes, above, and measurements of hexose uptake, above.

The antibodies were characterized by Western blot using membrane fractions prepared from HEK293T cells transfected with GLUTX1. No reactive bands were detected in mock-transfected cells, whereas two prominent bands were detected in GLUTX1-expressing cells: a laddering band extending from approximately 37 to 50 kDa, and a band with a molecular mass of greater than 70 kDa. The same two bands were seen using either the middle loop or the carboxyl-terminal antibodies. After treatment of the membranes with PNGaseF, the 37-50-kDa

band shifted toward a well defined band centered at approximately 35 kDa. The migration of the upper band was, however, only slightly affected. This band may represent nonspecific dimer aggregates of the lower band, because it is also sometimes visible in the reticulocyte lysates, and its intensity relative to the 37-50-kDa band depends in part on the cell lysis conditions, in particular on the addition of reducing agents.

To determine the cellular localization of GLUTX1, its cDNA was transiently expressed in HEK293T fibroblasts, and immunostaining was performed using the affinity-purified antibody directed against the cytoplasmic loop of GLUTX1. GLUTX1 showed a strong intracellular staining but no surface expression. Because mutation of the amino-terminal tail dileucine motif to alanines leads to detection of transport activity when the corresponding mRNA was injected into oocytes, we expected that the same mutant would be expressed at the cell surface. Results showed that most of the GLUTX1(LL-AA) is expressed on plasma membrane of transfected HEK293T cells. This therefore confirms the importance of this signal on the intracellular retention of GLUTX1.

GLUTX1 immunolocalization was analyzed by in situ hybridization in the adrenal medulla of the rat. Little to no GLUTX1 staining was found in the rat adrenal cortex. In the adrenal medulla, cytoplasmic structures, which stained for GLUTX1, localized to what appear to be nerve endings innervating chromaffin cells. Excitation of these nerves triggers catecholamines release from the chromaffin cells. In addition, GLUTX1 was innumolocalized to the pituitary. GLUTX1 colocalization with vasopressin but not oxytocin in these cells. GLUT2 was shown by immunolocalization to be expressed in nerves and glial cells of the brain, including glial cells, glial cells in the arcuate nucleus, and pyramidal cells.

Methods: Antibodies were raised against fusion proteins consisting of glutathione *S*-transferase and either the middle loop (amino acids 203-257) or the carboxyl-terminal tail (amino acids 459-478) of rat GLUTX1. Preparation of fusion proteins by PCR and immunization of rabbits were as described previously. See, *e.g.*, Widmann *et al.* 1995 *Biochem. J.* 310: 203-214. Affinity purification of the antibodies was on immobilized fusion proteins consisting of maltose binding protein, and the same regions of rat GLUTX1 were used for immunization. See, *e.g.*, Widmann *et al.* 1995 *Biochem. J.* 310: 203-214.

Example 6. Therapeutic uses of GLUTX compositions

The present invention may have direct bearing on the molecular causes of activity-dependent increase in glucose metabolism by brain neurons, in the normal situation, in ischemia and in the development of neurological pathologies, including neurodegenerative

diseases. Therefore, GLUTX polypeptides, nucleic acids and antibodies, as well as fragments, derivatives homologs and analogs thereof, can be used in the diagnosis of these diseases if genetic mutations can be identified as causes of these diseases.

In addition, endogenous GLUTX polypeptides can be used as a drug target by which small molecules or peptides can induce the surface expression of these transporter to stimulate glucose metabolism, either in the normal or diseased state.

GLUTX compositions can be used in gene therapy of diseases of the central nervous system. For instance, they can be engineered in lentiviral vectors that represent the tool of choice for transfer of genes into neurons. GLUTX2 and/or GLUT3 can thus be reexpressed at a normal level or overexpressed to facilitate glucose metabolism by damaged cells.

EQUIVALENTS

From the foregoing detailed description of the specific embodiments of the invention, it should be apparent that particular novel compositions and methods involving nucleic acids, polypeptides, antibodies, detection and treatment have been described. Although these particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims that follow. In particular, it is contemplated by the inventors that various substitutions, alterations, and modifications may be made as a matter of routine for a person of ordinary skill in the art to the invention without departing from the spirit and scope of the invention as defined by the claims. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. An isolated nucleic acid molecule encoding GLUTX, said molecule comprising a nucleotide sequence encoding a polypeptide having a sequence that is at least 85% identical to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21, or the complement of said nucleic acid molecule.

2. The nucleic acid molecule of claim 1, wherein said nucleotide sequence encodes a polypeptide of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21, or the complement of said nucleic acid molecule.

3. The nucleic acid molecule of claim 1, said molecule encoding the human GLUTX of SEQ ID NO: 1, 3, 5, 7, 9, 11 or 13, or the complement of said nucleic acid molecule.

4. The isolated nucleic acid molecule of claim 1, said molecule hybridizing under stringent conditions to a nucleic acid sequence complementary to a nucleic acid molecule comprising the sequence of nucleotides of SEQ ID NO: 1, 3, 5, 7, 9, 11 or 13, or the complement of said nucleic acid molecule.

5. The isolated nucleic acid molecule of claim 1, said molecule encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21, or an amino acid sequence comprising one or more conservative substitutions in the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21.

6. An oligonucleotide less than 100 nucleotides in length and comprising at lease 6 contiguous nucleotides of SEQ ID NO: 1, 3, 5, 7, 9, 11 or 13, or a complement thereof.

7. A nucleic acid vector comprising the nucleic acid molecule of claim 1.

8. The nucleic acid vector of claim 7, wherein said vector is an expression vector.

9. The vector of claim 7, further comprising a regulatory element operably linked to said nucleic acid molecule.

10. A host cell comprising the isolated nucleic acid molecule of claim 1.

11. An isolated GLUTX polypeptide at least 80% identical to a polypeptide selected from the group consisting of:

a) a polypeptide comprising an amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21;

b) a fragment of a polypeptide comprising an amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21, wherein the fragment comprises at least 6 contiguous amino acids of SEQ ID NO:2;

c) a derivative of a polypeptide comprising an amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21;

d) an analog of a polypeptide comprising an amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21;

e) a homolog of a polypeptide comprising an amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21;

f) A variant GLUTX polypeptide from SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21, wherein the amino terminal domain of said polypeptide does not include consecutive leucine residues; and

g) a naturally occurring allelic variant of a polypeptide comprising an amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21; wherein the polypeptide is encoded by a nucleic acid molecule that hybridizes to a nucleic acid molecule of SEQ ID NO: 1, 3, 5, 7, 9, 11 or 13 under stringent conditions.

12. The polypeptide of claim 11, wherein the polypeptide has a hexose transport activity.

13. An antibody that selectively binds to the polypeptide of claim 11, and fragments, homologs, analogs, and derivatives of said antibody.

14. A method of producing the polypeptide of claim 11, said method comprising the step of culturing the host cell of claim 10 under conditions in which the nucleic acid molecule is expressed.

15. A method of detecting the presence of the polypeptide of claim 11 in a sample, comprising contacting the sample with a compound that selectively binds to the polypeptide of claim 11 and determining whether the compound bound to the polypeptide of claim 11 is present in the sample.

16. A method of detecting the presence of a nucleic acid molecule of claim 1 in a sample, the method comprising contacting the sample with a nucleic acid probe or primer that selectively binds to the nucleic acid molecule and determining whether the nucleic acid probe or primer bound to the nucleic acid molecule of claim 1 is present in the sample.

17. A method for modulating the activity of the polypeptide of claim 11, the method comprising contacting a cell sample comprising the polypeptide of claim 11 with a compound that binds to said polypeptide in an amount sufficient to modulate the activity of the polypeptide.

18. A method of treating or preventing a disorder, said disorder being a hexose transport disorder, said method comprising administering to a subject in which such treatment or prevention is desired an amount of a therapeutic selected from the group consisting of:

a) the nucleic acid of claim 1;

b) the polypeptide of claim 11; and

c) the antibody of claim 13;

wherein said therapeutic is administered in an amount sufficient to treat or prevent hexose transport disorder in said subject.

19. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of a therapeutic selected from the group consisting of:

a) the nucleic acid of claim 1;

b) the polypeptide of claim 11; and

c) the antibody of claim 13;

and a pharmaceutically acceptable carrier.

20. A kit comprising in one or more containers, a therapeutically or prophylactically effective amount of the pharmaceutical composition of claim 19.

21. The use of a therapeutic in the manufacture of a medicament for treating a syndrome associated with a human disease, the disease being a hexose transport disorder, wherein said therapeutic is selected from the group consisting of:

a) the nucleic acid of claim 1;

b) the polypeptide of claim 11; and

c) the antibody of claim 13.

22. A method for screening for a modulator of activity or of latency or predisposition to hexose transport disorder, said method comprising:

a) administering a test compound to a test animal at increased risk for any one of a proliferative disorder, a differentiative disorder, and a glia-associated disorder, wherein said test animal recombinantly expresses a GLUTX protein;

b) measuring expression the activity of said protein in said test animal;

c) measuring the activity of said protein in a control animal that recombinantly expresses said protein and is not at increased risk for any one of a proliferative disorder, a differentiative disorder, and a glia-associated disorder; and

d) comparing expression of said protein in said test animal and said control animal, wherein a change in the activity of said protein in said test animal relative to said control animal indicates the test compound is a modulator of latency of any one of a proliferative disorder, a differentiative disorder, and a glia-associated disorder.

23. The method of claim 22, wherein said test animal is a recombinant test animal that expresses a test protein transgene or expresses said transgene under the control of a promoter at an increased level relative to a wild-type test animal, and wherein said promoter is not the native gene promoter of said transgene.

24. A method for determining the presence of or predisposition to a disease associated with altered levels of a GLUTX polypeptide of claim 11, the method comprising:

a) measuring the amount of the polypeptide in a sample from the mammalian subject; and

b) comparing the amount of said polypeptide in step (a) to the amount of the polypeptide present in a control sample,

wherein an alteration in the level of the polypeptide in step (a) as compared to the control sample indicates a disease condition.

25. A method for determining the presence of or predisposition to a disease associated with altered levels of a GLUTX nucleic acid of claim 1, the method comprising:

a) measuring the amount of the nucleic acid in a sample from the mammalian subject; and

b) comparing the amount of said nucleic acid in step (a) to the amount of the nucleic acid present in a control sample,

wherein an alteration in the level of the nucleic acid in step (a) as compared to the control sample indicates a disease condition.

26. A method of treating a pathological state in a mammal, the method comprising administering to the subject a polypeptide to a subject in an amount to alleviate the pathological condition, wherein the polypeptide a polypeptide having an amino acid sequence at least 85% identical to a polypeptide with an amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 15, 16, 17, 18, 19, 20 or 21, or a biologically active fragment thereof.

27. A method of treating a pathological state in a mammal, the method comprising administering to the subject the antibody of claim 13 in an amount sufficient to alleviate the pathological condition.

28. The method of claim 26, wherein the polypeptide is targeted to the brain.

29. The method of claim 26, wherein the polypeptide is targeted to the testes.

30. The method of claim 26, wherein the pathological state is any one hexose transport disorder selected from the group comprising ischemia, diabetes, hyperglycemia, hypoglycemia, a glucose metabolism disorder, and a neurodegenerative disease.

FIG. 1

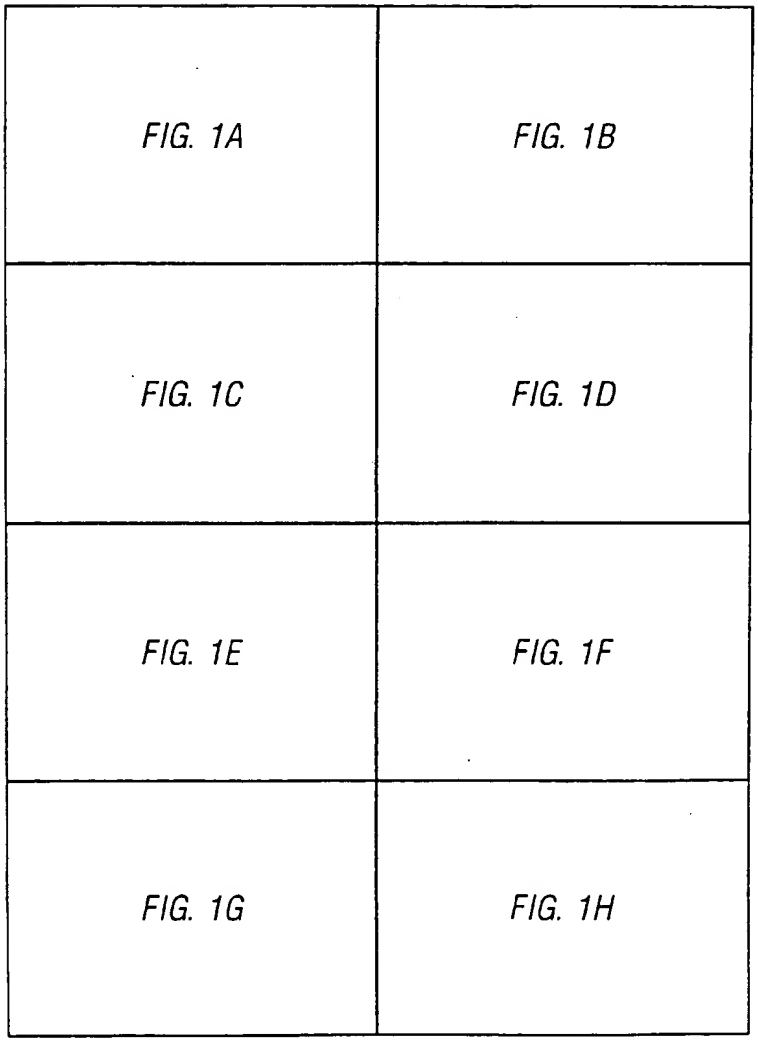
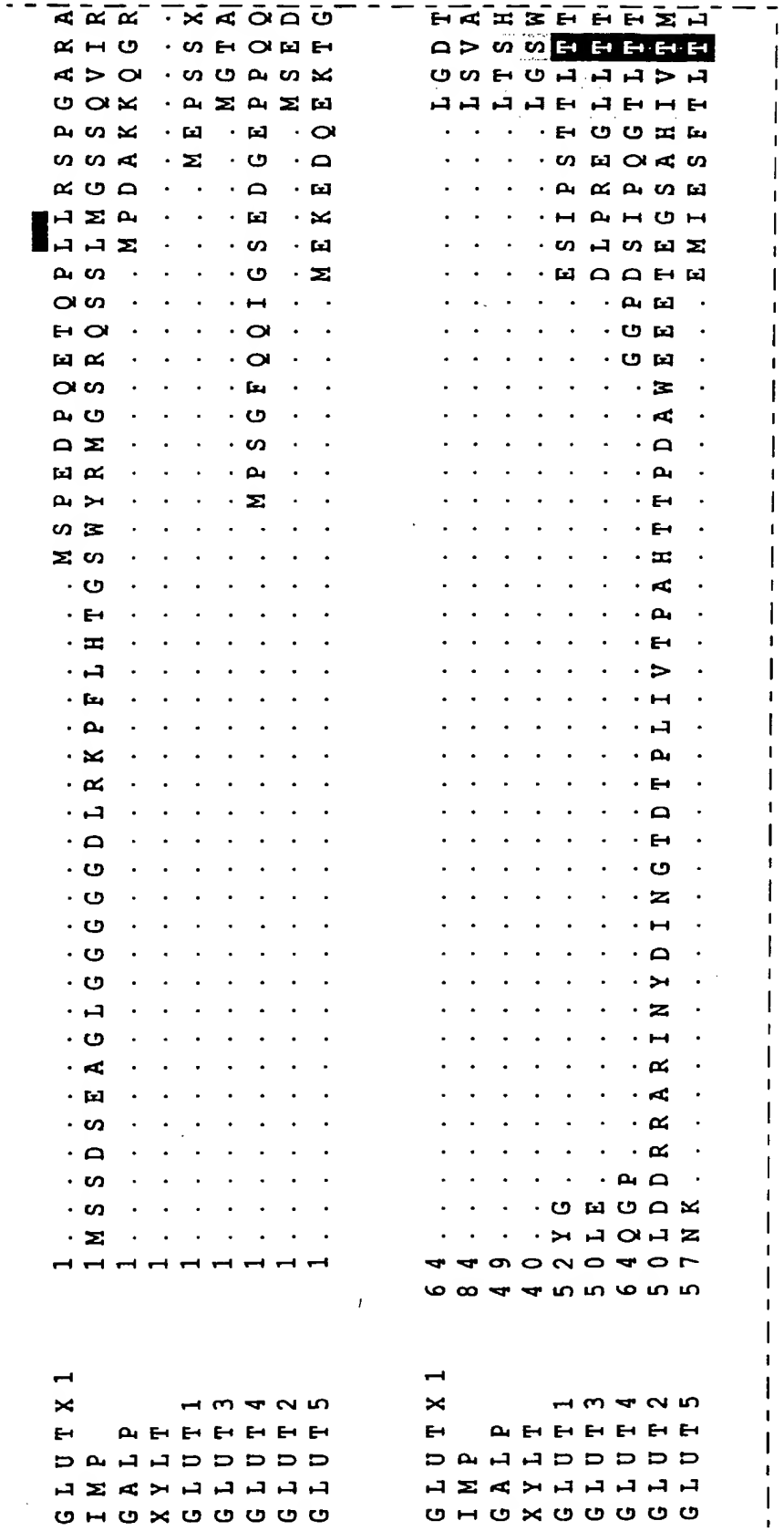


FIG. 1A



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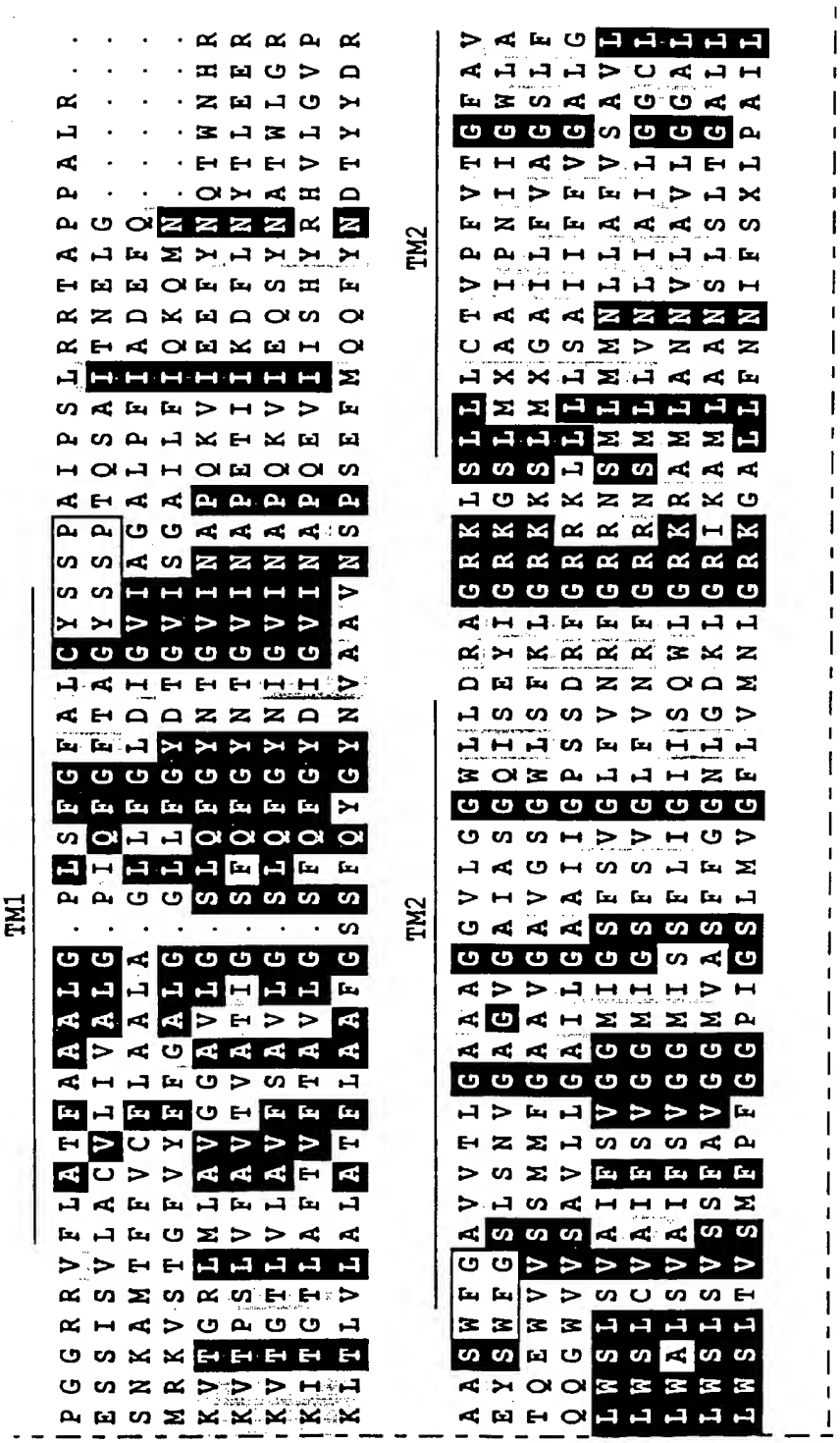


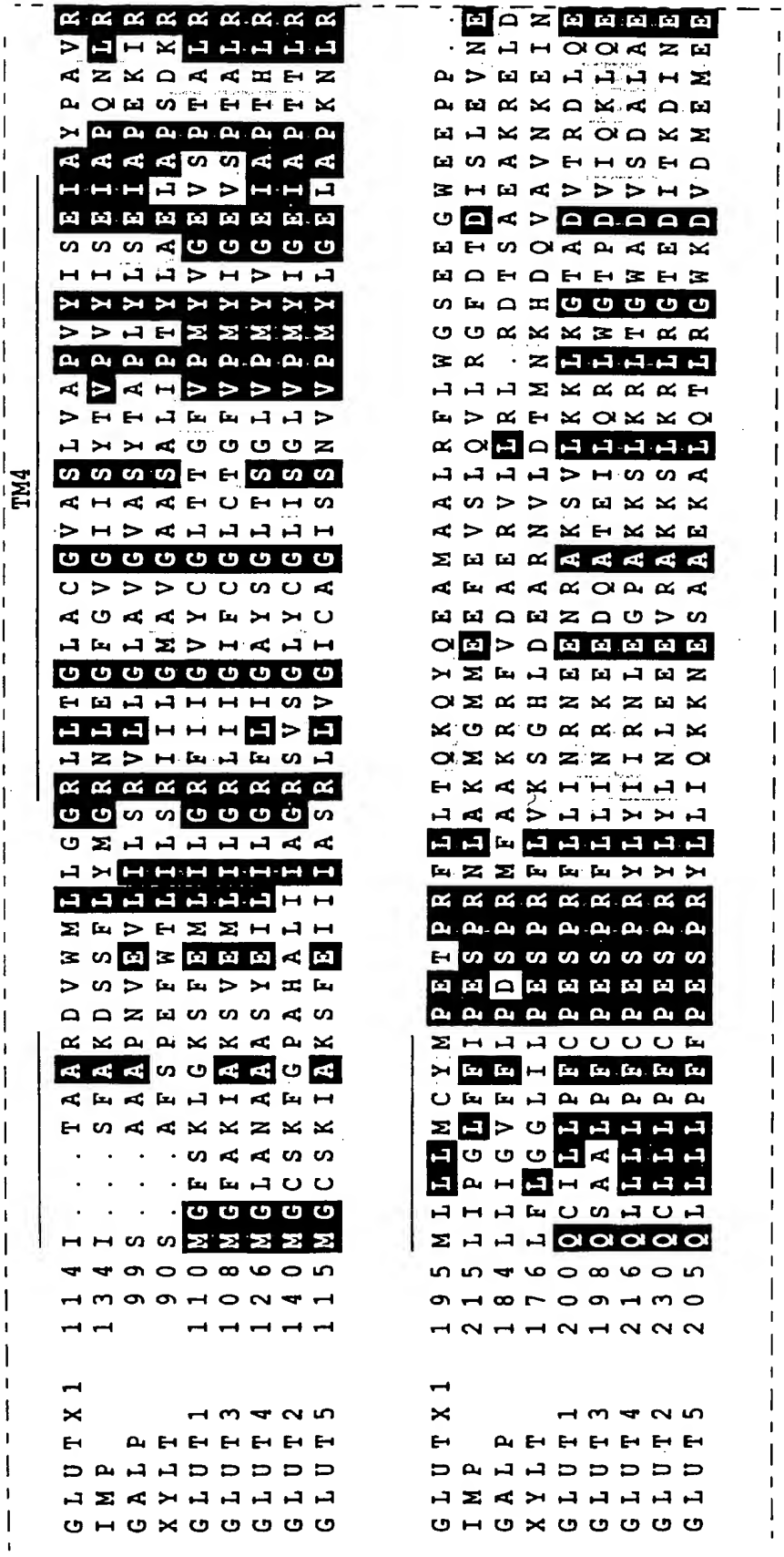


FIG. 1C

FIG. 1D

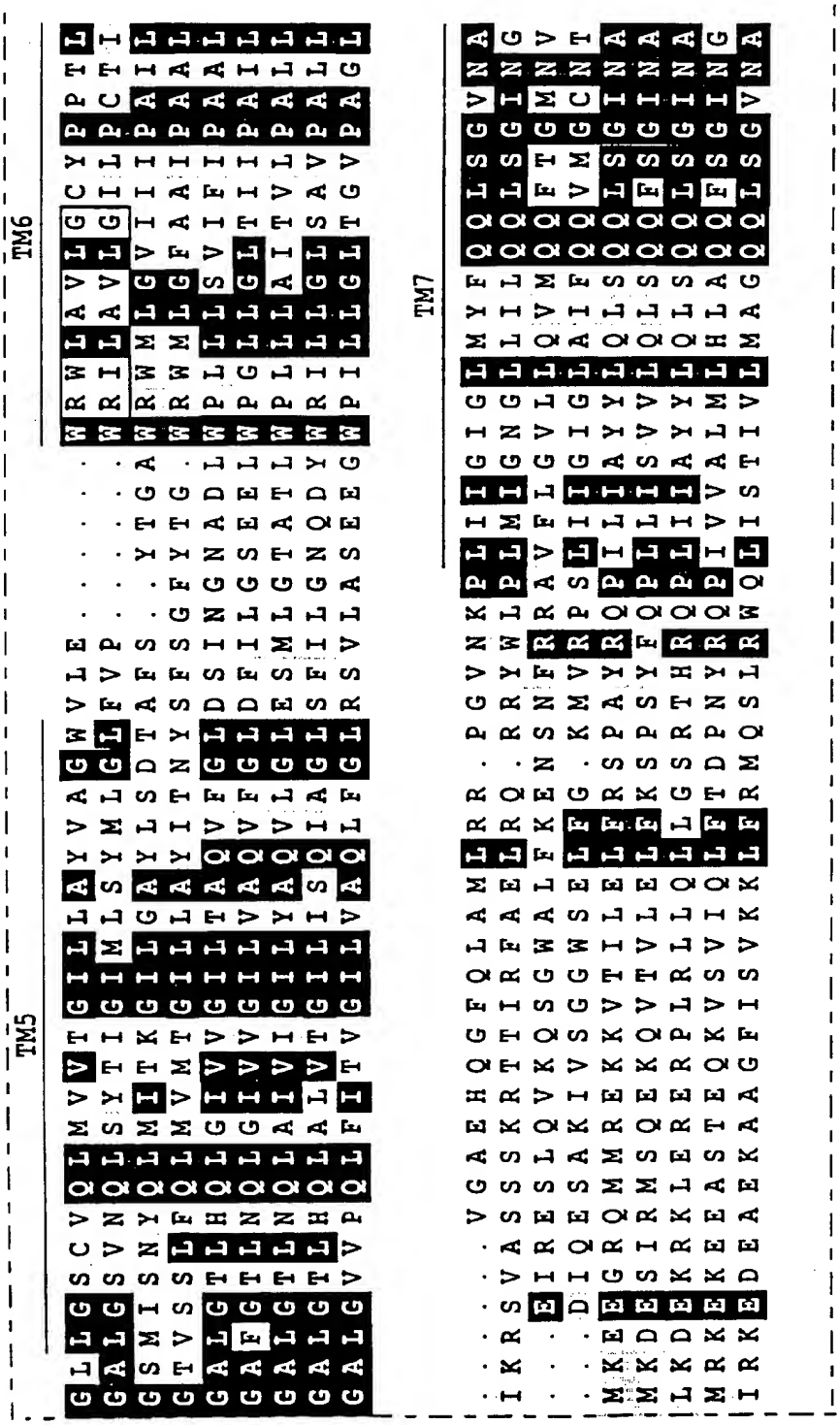


FIG. 1E

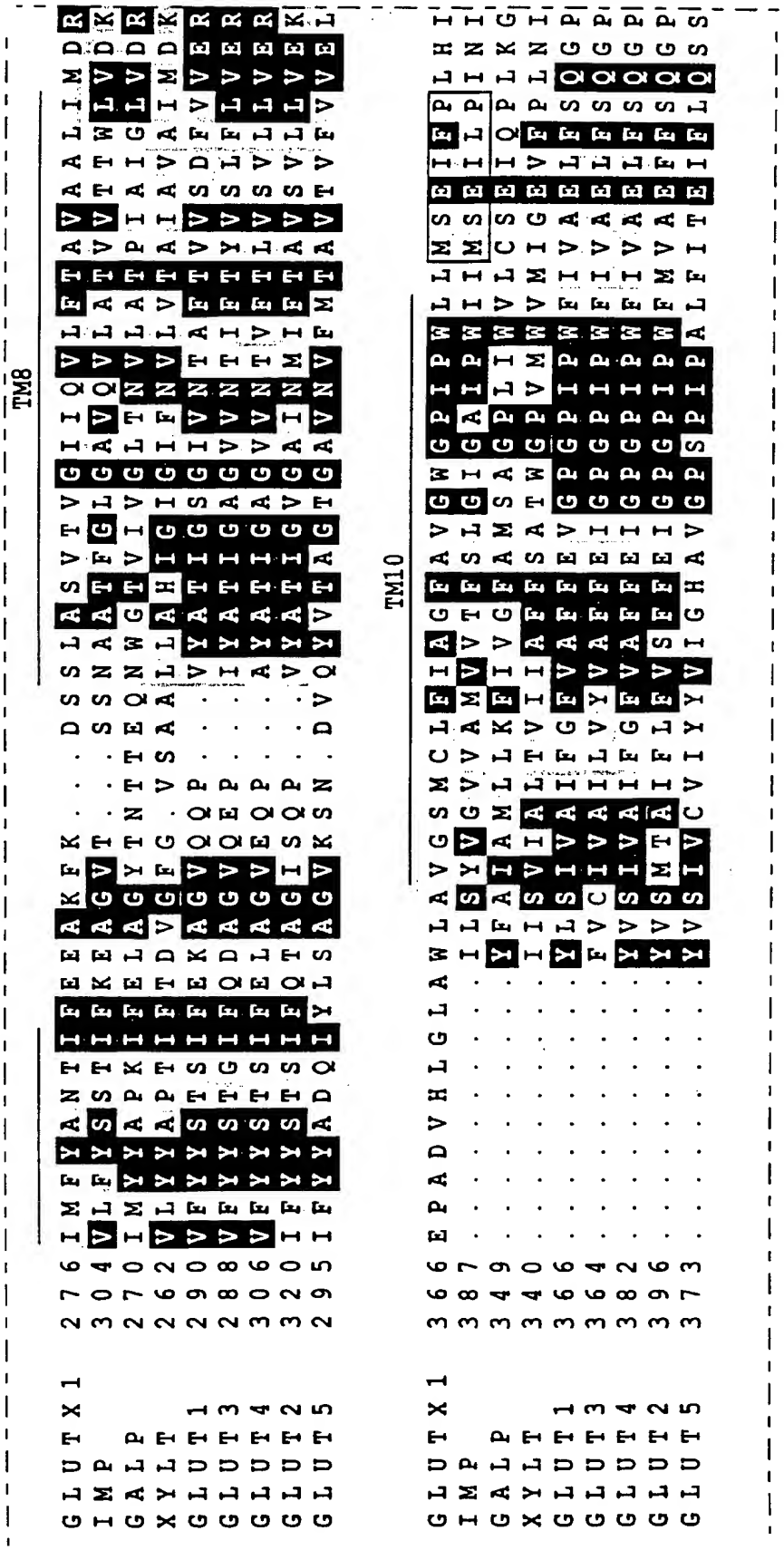
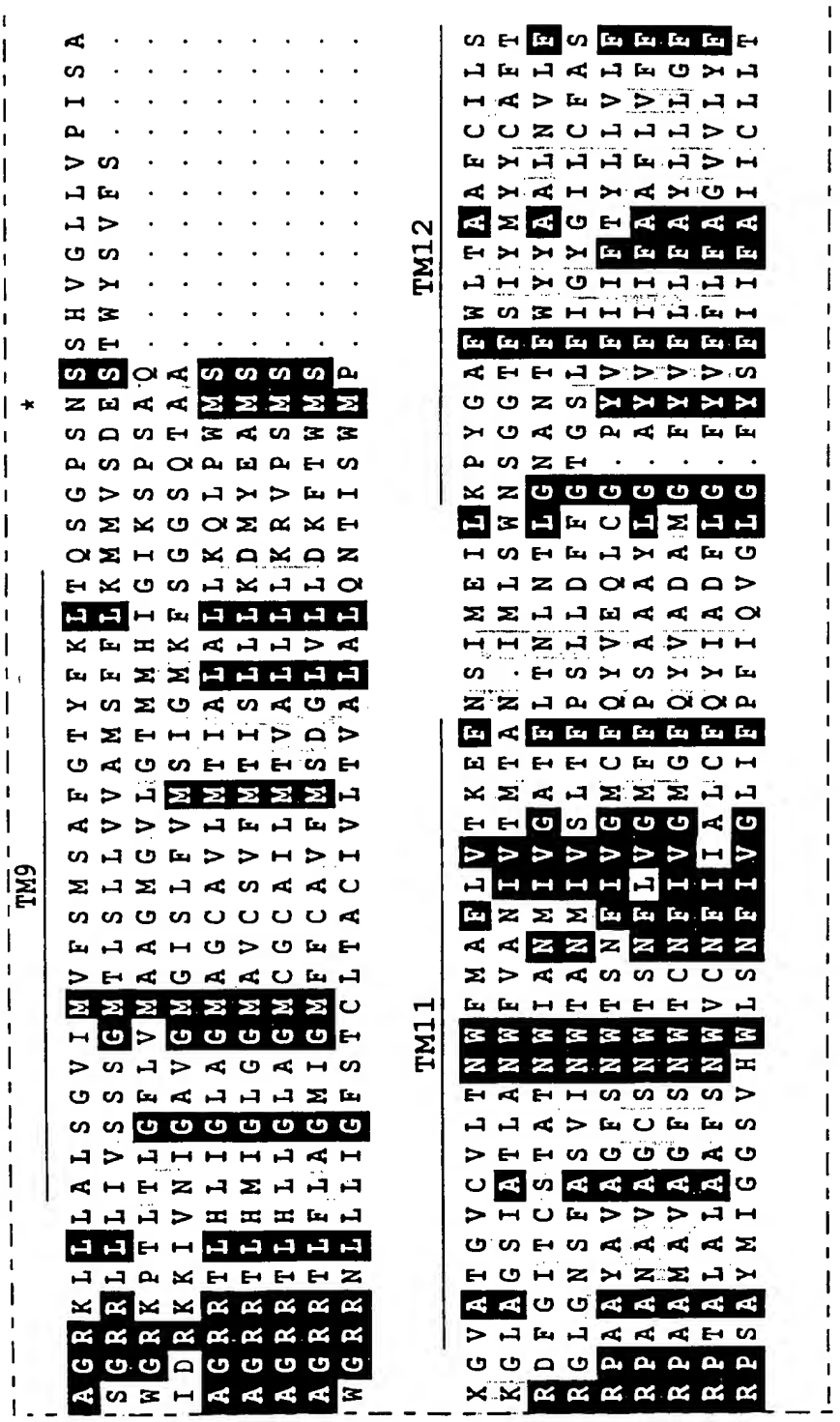


FIG. 1F



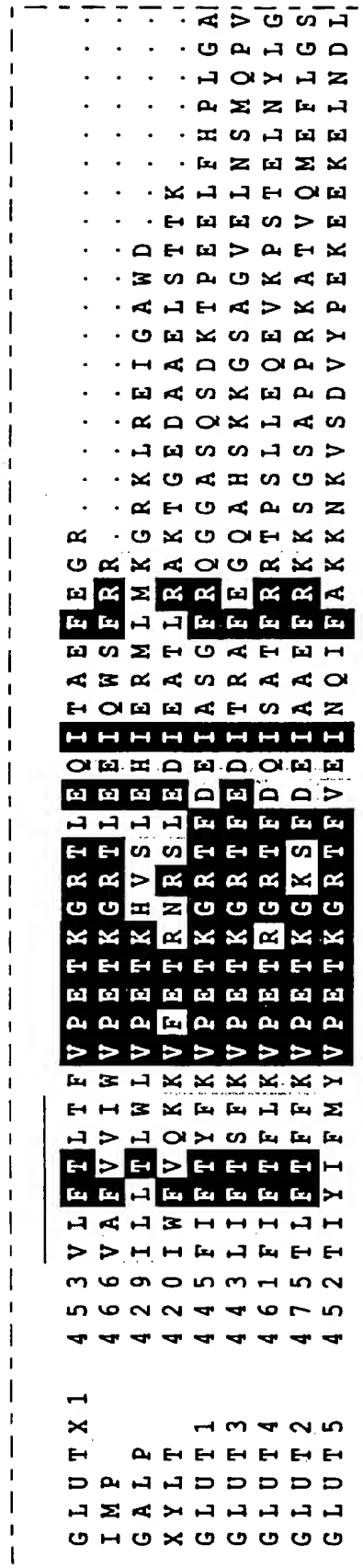


FIG. 1G

FIG. 1H

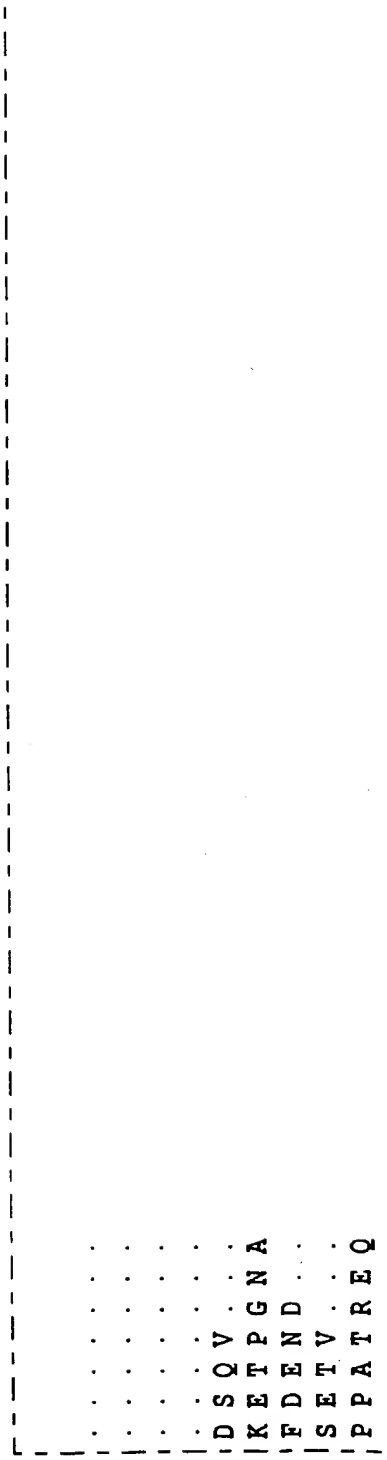


FIG. 2 (PANEL A)

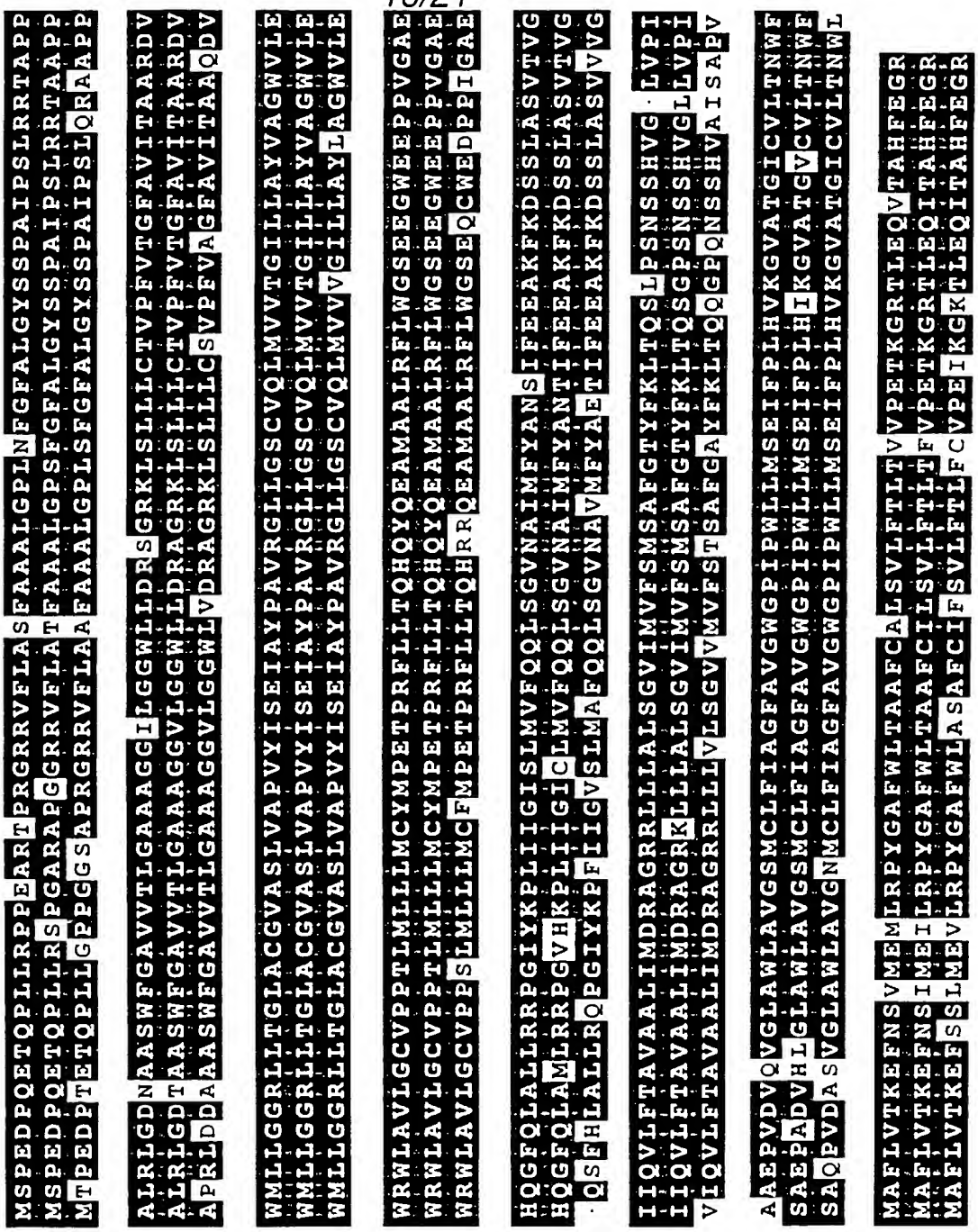
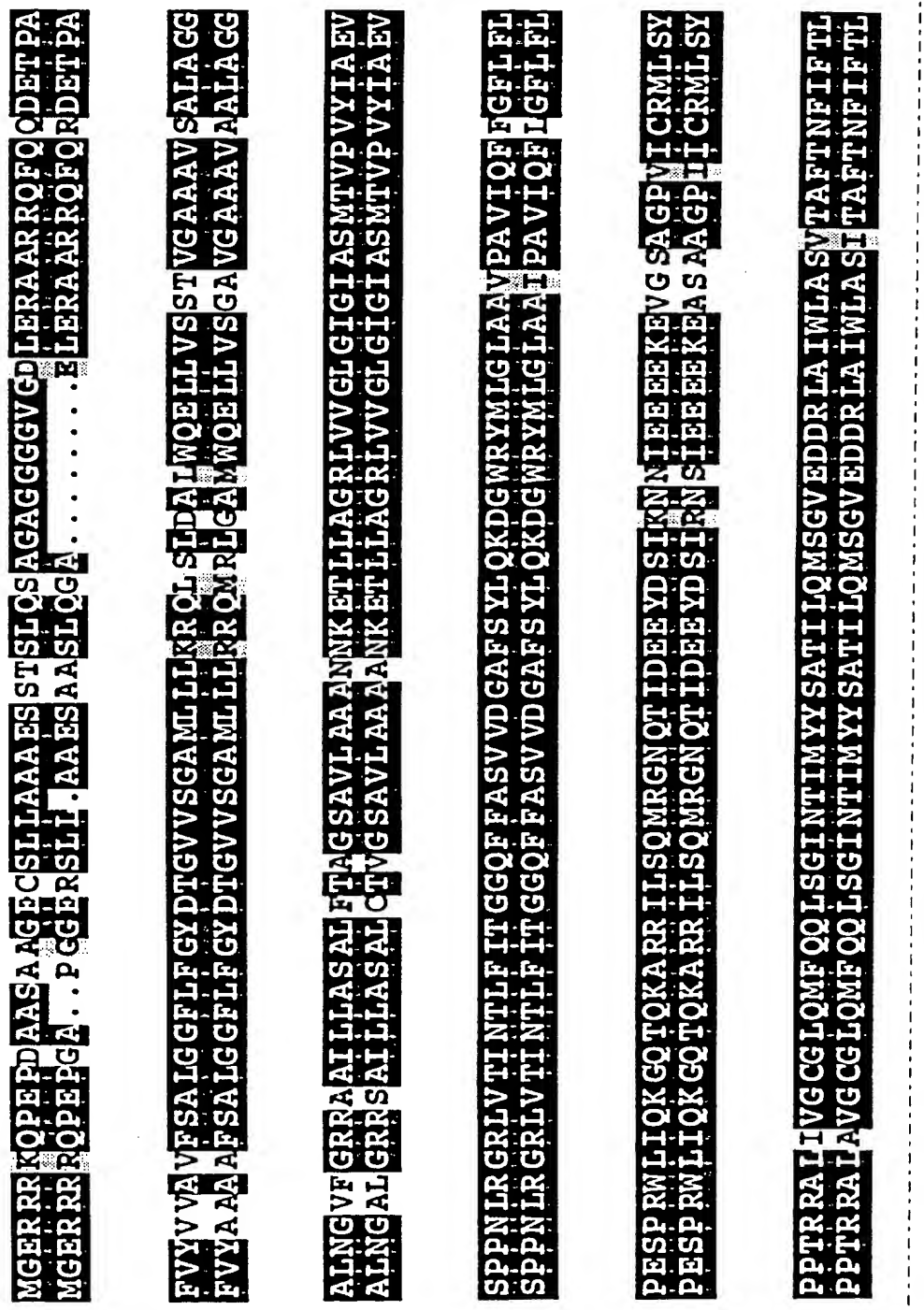

GLUTX1 MOUSE 1
GLUTX1 RAT 1
GLUTX1 HUMAN 1
GLUTX1 MOUSE 61
GLUTX1 RAT 61
GLUTX1 HUMAN 61
GLUTX1 MOUSE 121
GLUTX1 RAT 121
GLUTX1 HUMAN 121
GLUTX1 MOUSE 181
GLUTX1 RAT 181
GLUTX1 HUMAN 181
GLUTX1 MOUSE 241
GLUTX1 RAT 241
GLUTX1 HUMAN 241
GLUTX1 MOUSE 301
GLUTX1 RAT 301
GLUTX1 HUMAN 300
GLUTX1 MOUSE 360
GLUTX1 RAT 361
GLUTX1 HUMAN 360
GLUTX1 MOUSE 420
GLUTX1 RAT 421
GLUTX1 HUMAN 420

FIG. 2 CONTD
(PANEL B)



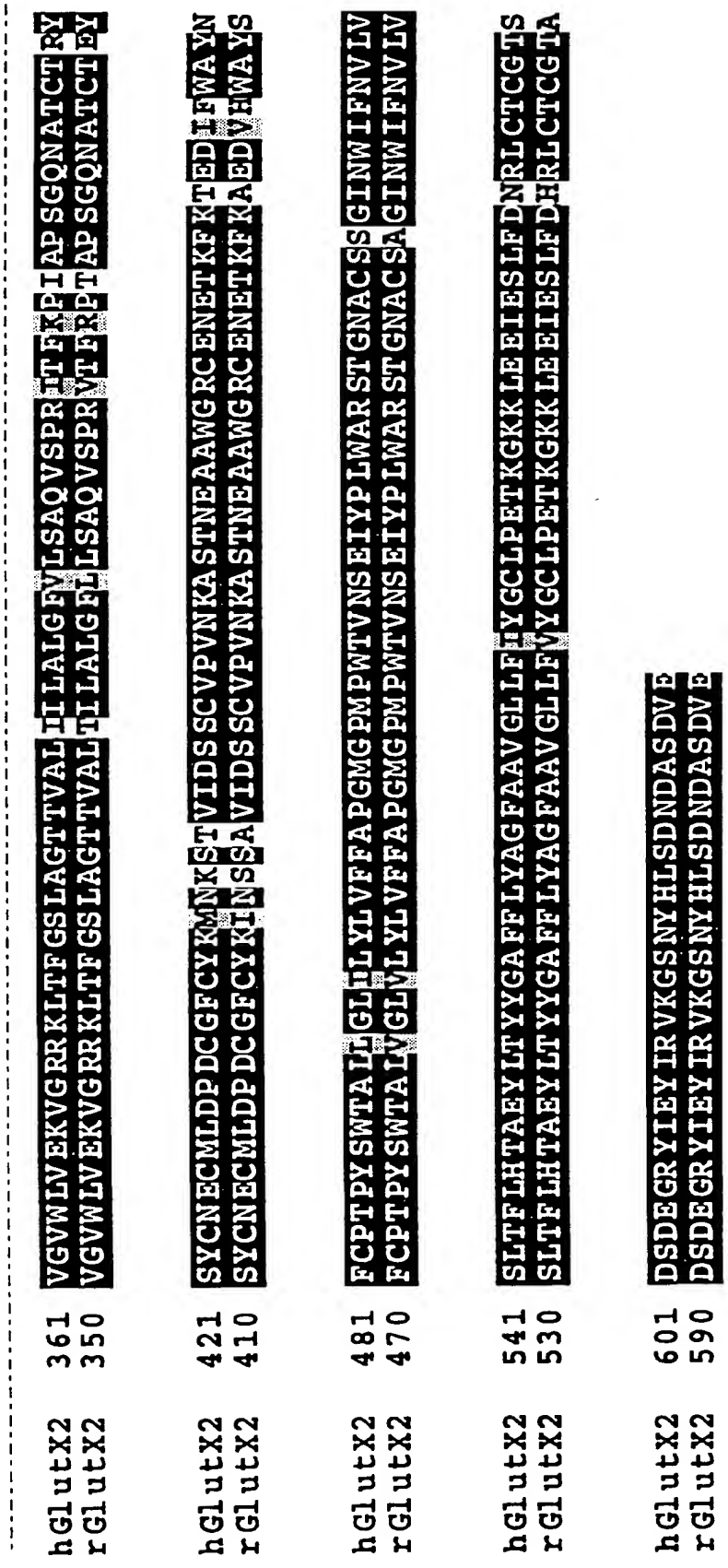


FIG. 2 CONTD
(PANEL B)

FIG. 3 (PANEL A)

```
glutx3human       -------MQEP----------LLGAEGPDYDTFPEKPPPSPGDRA----RVGTLQNKR--
glutx1human       ------MTPEDP----EETQPLL---GP---------PGGS----APRG-RR---------
glutx2human       MGERRRKQPEPDAASAAGECSLLAAAESSTSLQSAGAGGGGVGDLERAARRQFQQDETPA
                            *           .**.*  ....  .  .  .  .  *  *    *:.

glutx3human       -VFLATFAAVLGNFSFGYALVYTSPVIPALERSLDPDLHLTKSQASWFGSVFTLGAAAGG
glutx1human       -VFLAAFAAALGPLSFGFALGYSSPAIPSLQRAAPPAPRLDDAAASWFGAVVTLGAAAGG
glutx2human       FVYVVAVFSALGGFLFGYDTGVVSGAMLLLKRQLS----LDALWQELLVSSTVGAAAVSA
                   *:::.:. :.** : **:     * .:  *:*       *     . : :  . .**...

glutx3human       LSAMILNDLLGRKLSIMFSAVPSAAGYAVMAGAHGLWMLLLGRTLTGFAGGLTAACIPVY
glutx1human       VLGGWLVDRAGRKLSLLLCSVPFVAGFAVITAAQDVWMLLGGRLLTGLACGVASLVAPVY
glutx2human       LAGGALNGVFGRRAAILLASALFTAGSAVLAAAANNKETLLAGRLVVGLGIGIASMTVPVY
                  : .  * .  **: ::::.:.  .** **::.*:.   ** ** :.*:. *:::   ***

glutx3human       VSEIAPPGVRGALGATPQLMAVFGSLSLYALG------LLLPWRWLAVAGXAPVLIMILL
glutx1human       ISEIAYPAVRGLLGSCVQLMVVVGILLAYLAG------WVLEWRWLAVLGCVPPSLMLLL
glutx2human       IAEVSPPNLRGRLVTINTLFITGGQFFASVVDGAFSYLQKDGWRYMLGLAAVPAVIQFFG
                  ::*:: * :** * :   *: . * :     .          **::   . .*  : ::

glutx3human       LSFMPNSPRFLLSRGRDEEALRALAWLRGTDVDVHWEFGQIQDNVRRQSSRVSWA-----
glutx1human       MCFMPETPRFLLTQHRRQEAMAALRFLWGSEQ--GWE-----DPPIGAEQSFHLA-----
glutx2human       FLFLPESPRWLIQKGQTQKARRILSQMRGNQT-IDEEYDSIKNNIEEEEKEVGSAGPVIC
                  : *:*::**:*: : : ::*   *  : *.:     *     :     .. .  *

glutx3human       -EARAPHVCRPITVALLMRLLQQLTGITPILVYLQSIFDSTAVLLP---PKDDAAIVGXV
glutx1human       -LLRQPGIYKPFIIGVSLMAFQQLSGVNAVMFYAETIFEEAKFKDS----SLASVVVGVI
glutx2human       RMLSYPPTRRALIVGCGLQMFQQLSGINTIMYYSATILQMSGVEDDRLA-IWLASVTAFT
                       *   :.: :.  :  :***:*:..:: *  :*:: : .          : :..
```

```
glutx3human     RLLSVLIAALTMDLAGRKVLLFVSAAIMFAANLTLGLYIHFGPRP--------LSPNS--
glutx1human     QVLFTAVAALIMDRAGRRLLLVLSGVVMVFSTSAFGAYFKLTQG----------GPG---
glutx2human     NFIFTLVGVWLVEKVGRRKLTFGSLAGTTVALIILALGFVLSAQVSPRITFKPIAPSGQN
                ..:  .  :..   :: .**: * . * .    :   :.  : :              .*.

glutx3human     ---------------------------TAGLESESWGDLAQPLAAPAGY-----------
glutx1human     ---------------------------NSSHVAISAPVSAQPVDASVG-------------
glutx2human     ATCTRYSYCNECMLDPDCGFCYKMNKSTVIDSSCVPVNKASTNEAAWGRCENETKFKTED
                                          .:   : .      .   ..

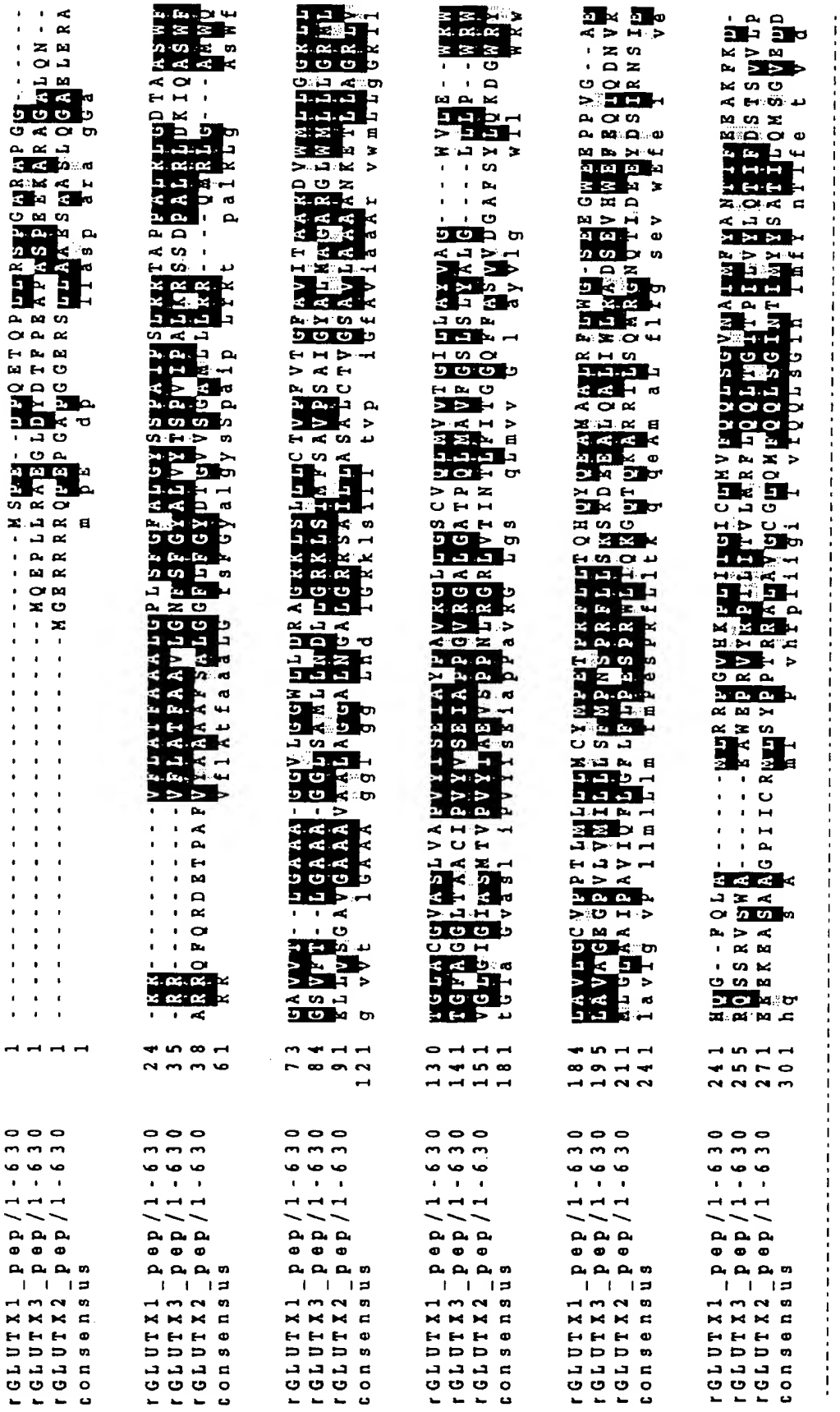
glutx3human     -----------LTLVPLLATMLFIMGYAVGWGPITWLLMSEVLPLRARGVASGLCVLASW
glutx1human     -----------LAWLAVGNMCLFIAGFAVGWGPIPWLLMSEIFPLHVKGVATGICVLTNW
glutx2human     IFWAYNFCPTPYSWTALLGLILYLVFFAPGMGPMPWTVNSEIYPLWARSTGNACSSGINW
                             :  .:    *::  :* * **:.* : **: ** .:..... .   .*

glutx3human     LTAFVLTKSFLPVVSTFGLQVPFLFFAAICLVSLVFTGCCVPETKGRSLEQIESFFRTGR
glutx1human     LMAFLVTKEFSSLMEVLRPYGAFWLASAFCIFSVLFTLFCVPEIKGKTLEQITAHFEG-R
glutx2human     IFNVLVSLTFLHTAEYLTYYGAFFLYAGFAAVGLLFIYGCLPETKGKKLEEIESLFDN-R
                :  .:::  *    . :    .* : :.:. ..::*   *:** **:.**:* : *   *

glutx3human     RSFLR-----------------------------------
glutx1human     ----------------------------------------
glutx2human     LCTCGTSDSDEGRYIEYIRVKGSNYHLSDNDASDVEX
```

FIG. 3 CONTD
(PANEL A)

FIG. 3 CONTD
(PANEL B)



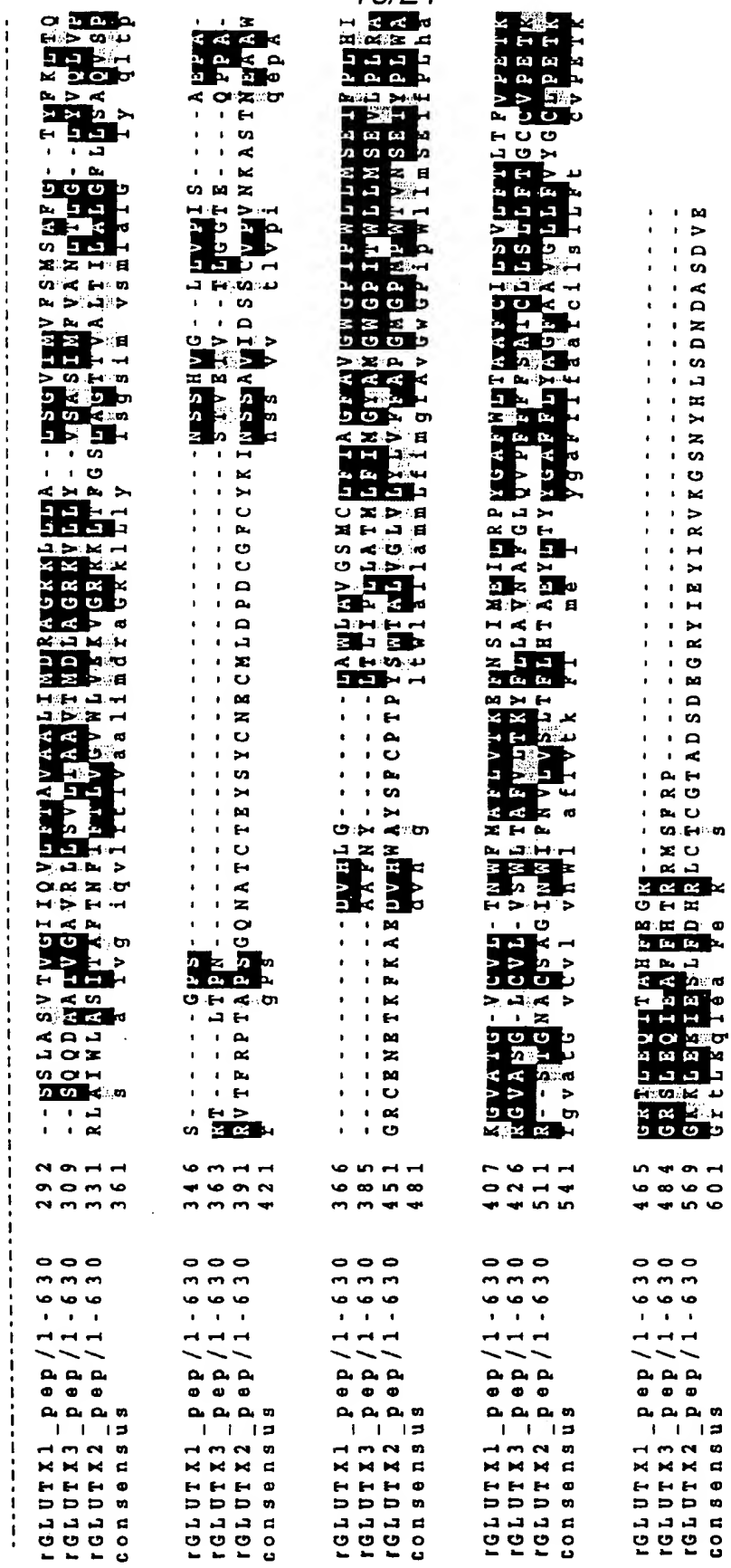


FIG. 3 CONTD
(PANEL B)

FIG. 4

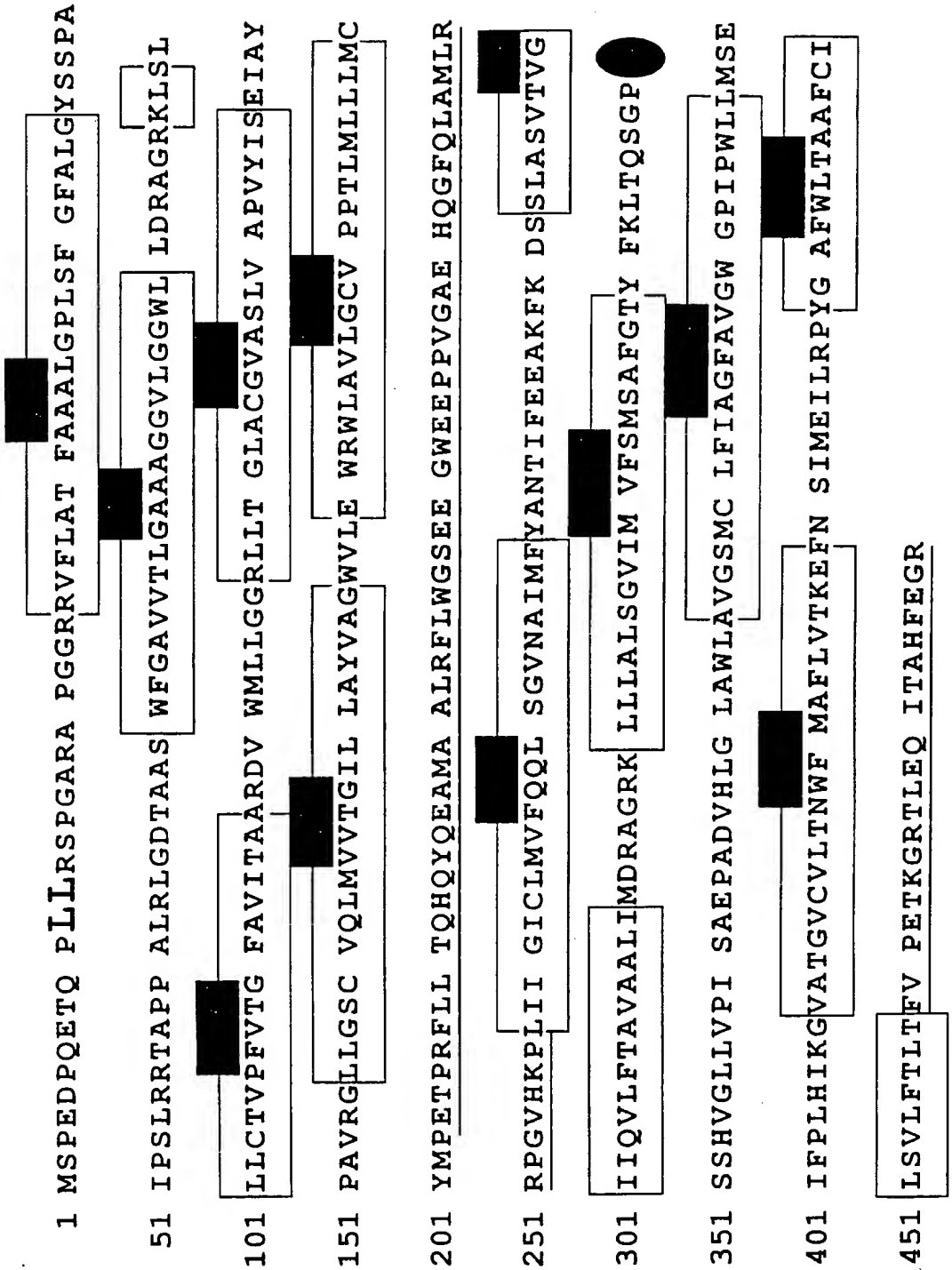

1 MSPEDPQETQ PLLRSPGARA PGGRRVFLAT FAAALGPLSF GFALGYSSPA
51 IPSLRRTAPP ALRLGDTAAS WFGAVVTLGAAAGGVLGGWL LDRAGRKLSL
101 LLCTVPFVTG FAVITAARDV WMLLGGRLLT GLACGVASLV APVYISEIAY
151 PAVRGLLGSC VQLMVVTGIL LAYVAGWVLE WRWLAVLGCV PPTLMLLLMC
201 YMPETPRFLL TQHQYQEAMA ALRFLWGSEE GWEEPPVGAE HQGFQLAMLR
251 RPGVHKPLII GICLMVFQQL SGVNAIMFYA NTIFEEAKFK DSSLASVTVG
301 IIQVLFTAVA ALIMDRAGRK LLLALSGVIM VFSMSAFGTY FKLTQSGP
351 SSHVGLLVPI SAEPADVHLG LAWLAVGSMC LFIAGFAVGW GPIPWLLMSE
401 IFPLHIKGVA TGVCVLTNWF MAFLVTKEFN SIMEILRPYG AFWLTAAFCI
451 LSVLFTLTFV PETKGRTLEQ ITAHFEGR

FIG. 5(A)

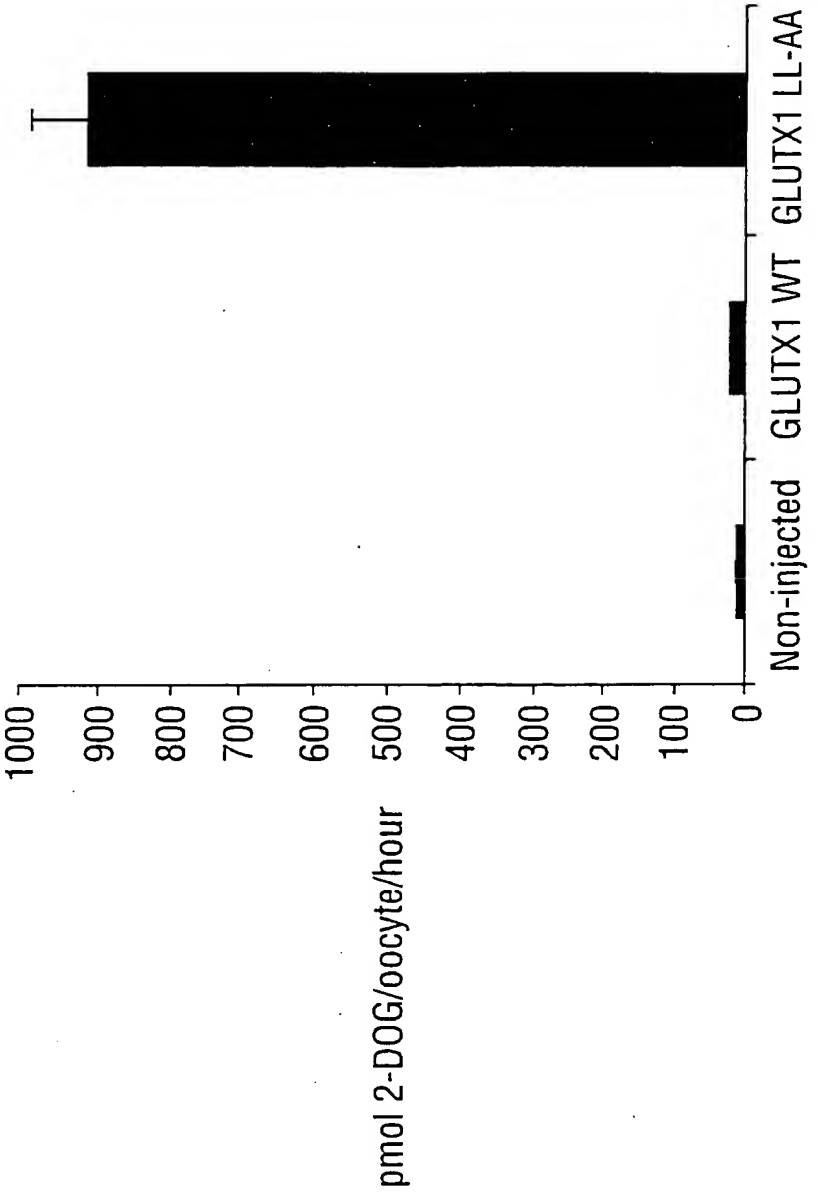

1000
900
800
700
600
500
400
300
200
100
0
pmol 2-DOG/oocyte/hour
Non-injected
GLUTX1 WT
GLUTX1 LL-AA

FIG. 5(B)

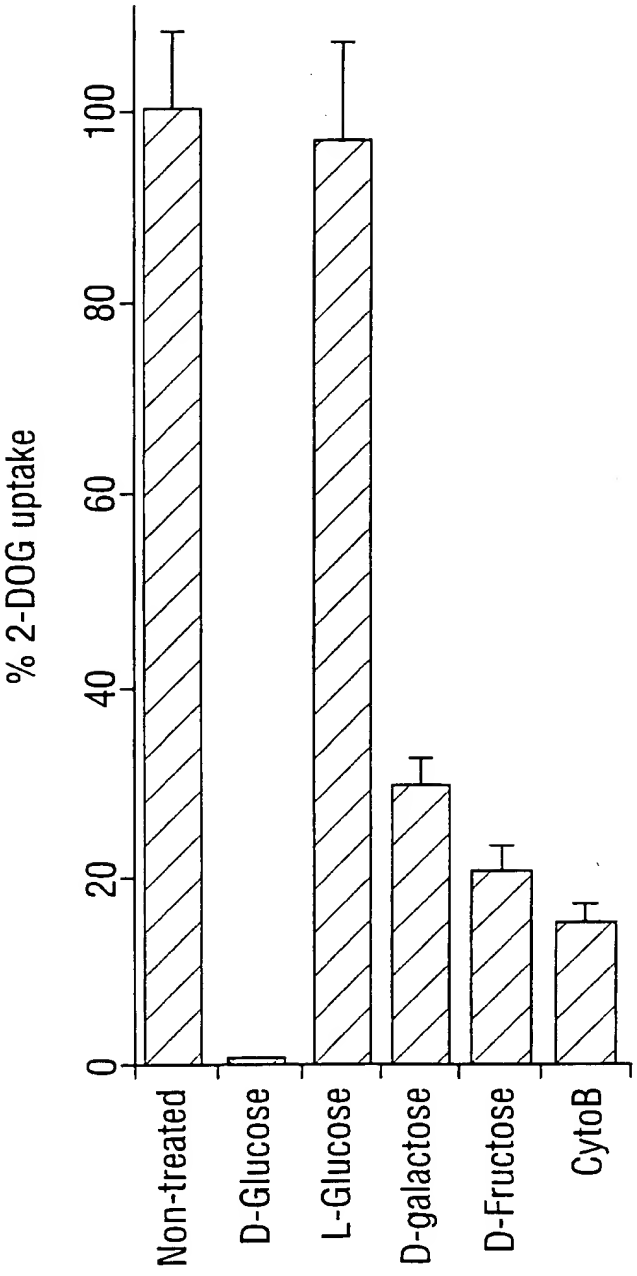

% 2-DOG uptake
0
20
40
60
80
100
Non-treated
D-Glucose
L-Glucose
D-galactose
D-Fructose
CytoB

FIG. 5(C)

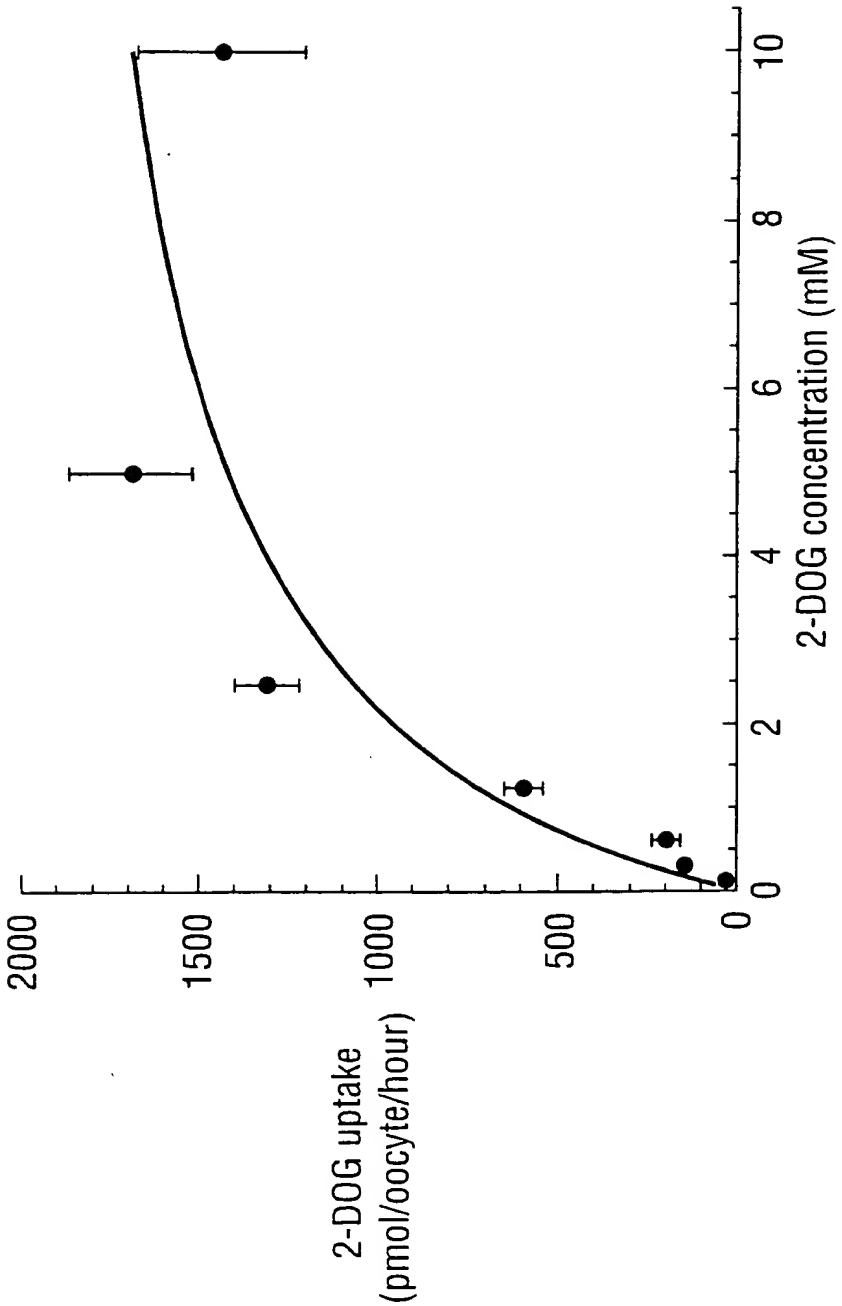
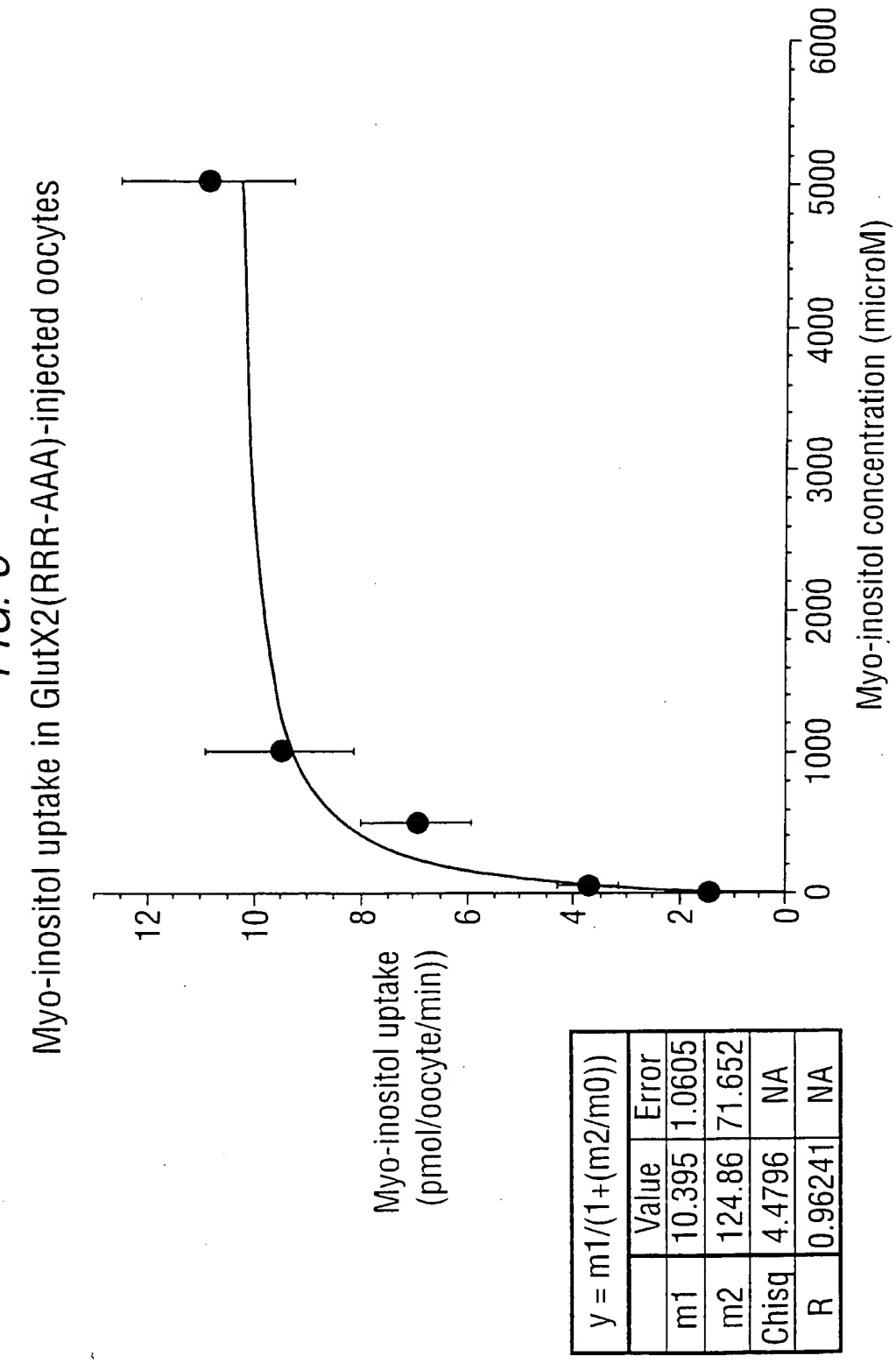

2-DOG uptake
(pmol/oocyte/hour)
2000
1500
1000
500
0
0
2
4
6
8
10
2-DOG concentration (mM)

FIG. 6



y = m1/(1+(m2/m0))		
	Value	Error
m1	10.395	1.0605
m2	124.86	71.652
Chisq	4.4796	NA
R	0.96241	NA